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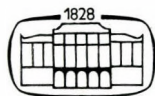
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PHYTOGEOGRAPHIC SURVEY OF CUBA

I. THE PHYTOGEOGRAPHIC CHARACTERISTICS AND EVOLUTION OF THE FLORA OF CUBA

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Some of the main characteristics of the flora of Cuba are exposed and discussed, as the dominance of endemics, disjunction, vicariance, inversion, microphyllia, micranthia, relict character and vulnerability. The origin, evolution and presumable migrations of the flora are also discussed, based on the results of the geological up-to-date investigations on the plate tectonics. In the process of the flora evolution three main phases are distinguished, the early plate phase, the middle-tertiary land-bridge phase and the late archipelago phase. As the last important period of the flora immigrations the middle tertiary land-bridge phase is discussed with its different floras, as the broad-leaved Honduras-flora, and the sclerophyllous Madro-Tethyan flora. In the late archipelago phase the broad-leaved Guyana-flora, the semideciduous Yucatan-flora and the extratropical North-American flora influenced the evolution of the flora and vegetation of Cuba. At last the evolution centres of the ecologically and/or phytosociologically adapted species groups and their migratory routes are designed and explained.

The phytogeographic characteristics of Cuba

Some outstanding phytogeographic features of Cuba

The floristic analysis and the study of chorological types suggest that the most important and most typical characteristics of the flora of Cuba are as follows: 1. Dominance of endemics, 2. Disjunction, 3. Vicariance, 4. Inversion, 5. Microphyllia, 6. Micranthia, 7. Relics, and 8. Vulnerability.

The dominance of endemics

As shown earlier (BORHIDI 1982), the endemics comprise a total of 51.4% of the native flora. This is outstandingly the largest percentage in the Antilles. MARIE-VICTORIN (1942 1944, 1956) attributed this to the high Mg and Fe concentrations of "limonite areas". According to ALAIN's (1958 p. 16) hypothesis, the reasons are the early isolation of the flora during the upper Miocene, the diverse edaphic soil conditions of the country and the arid climate of certain areas.

The ecology of endemic speciation

In examining the ecological background of the speciation of endemics MUÑIZ (1970) found that eight habitat types have substantial influence on flora development. These habitats occur in 25 areas in Cuba. The habitat types with the number of areas in brackets are:

- A. Ferritic soils and tropical brown soils derived from serpentine (6)
- B. Ferralitic soils derived from serpentine (5)
- C. Oligotrophic quartz-allitic yellow soils (2)
- D. White sandy habitats (2)
- E. Young, arid, coastal limestone areas (4)
- F. Young, montane, karstic limestone areas (2)
- G. Old, montane limestone karst, "haystack mountains" (1)
- H. High montane areas

The geographic distribution of these habitats is shown in the Atlas Nacional of Cuba p. 60. No doubt that the number of factors facilitating the speciation of endemics is large. The influence and importance of these factors differed with time and space. Factors potentially influencing speciation are listed below:

- A. Insularity and isolation
 - a. Geographic factors
 - external (insularity)
 - internal (isolation)
 - b. Ecological factors
 - ba. Orographic factors
 - alternation of lowlands and mountains
 - great relief-energy
 - bb. Geological and edaphic factors
 - serpentine and other ultrabasic rocks
 - frequent occurrence of limestone karsts
 - frequent occurrence of acid, slatey bedrocks
 - frequent occurrence of acid, white sands
 - bc. Sociological factors
 - community mosaics
 - communities as barriers of migration
 - interactions between plants and animals
- B. Climatic changes
 - c. Alternation of wet and dry periods
 - d. Alternation of cool wet and warm dry periods
- C. Genetic factors
 - e. Mutagenic speciation
 - f. Hybridogenic speciation, introgression
 - g. Genetic drift

Horizontal distribution of endemics

The study of the horizontal and vertical distribution of endemics yields valuable information on the ecological effects influencing speciation and geographic range. Figure 1 shows the number of endemic species per area. The intertidal mangroves and mangrove swamps are the poorest in endemics. The lowlands of central Cuba and the southern part of Isla de Pinos are moderately poor, having 25–50 endemic species. Moderately rich areas (50–75) are the younger coastal limestone habitats (Guanahacabibes peninsula, the southern coast of Las Villas and the northwestern coast of Oriente), the slatey outcrops and white sands in Isla de Pinos, the hilly regions of Central Cuba and the medium altitude zone of Sierra Maestra. The rich areas, containing 76–100 regional endemics, are the slatey outcrops of Pinar del Rio, the ancient serpentines of Cajalbana, the younger serpentine zones of Habana,

Matanzas and Motembo, the rainforest region of Sierra Escambray, the southern coast at Sierra Maestra and the northern karstic zone (Guisa-Baire) in Oriente, and the limestones of the Sagua-Baracoa massif. Most areas very rich in endemics (100–150) are on serpentine, three are younger (Santa Clara, Camagüey, Holguin) and three others are ancient latosol areas (Nipe, Cristal, Moa-Baracoa). In respect of endemic richness only the ancient karsts with conical formations in the Sierra de los Organos, the montane zone of Sierra Maestra and the xerotherm semi-deserts of southern Baracoa are commensurable to the above localities. Outstandingly rich areas are found only in the montane zone of ancient serpentine mountains, such as the Nipe Mts., the surroundings of Pico del Cristal and the highlands of Moa (El Toldo, Iberia, etc.), where more speciation-inducing factors (age, serpentine, isolation, montane character) are combined.

Vertical distribution of endemics

After reading the foregoing one would expect that the number of endemics increases as altitude increases, due to the montane effects. However, as our studies in Pico Turquino showed, the vertical distribution of endemics is a more complex matter. Figure 2 demonstrates that the number of endemics increase up to the lower limit of the cloud zone and then rapidly decreases. Its reason is that the vertical distribution of regional endemics of lowlands and mountains of medium height differs from that of the local montane endemics. The number of species from the first group increases up to 1000 m, hardly changes up to 1500 m and then falls on a sudden. The number of montane endemics, however, abruptly increases between 1000 and 1500 m and does not change any more. The disappearance of lowland regional endemics from the high altitude zone causes the decrease of endemic species (Fig. 2/A). Contrary to the absolute figures, the relative proportion of endemics rapidly increases with altitude. That is, the few endemics occurring become predominant at higher elevations (Fig. 2/B).

Relationship between aridity and the number of endemics

STEBBINS (1953) pointed out that ecological drought stimulates speciation. The ecological and chorological study of many Cuban endemics provides evidence for this phenomenon. The largest number and highest density of endemics may be observed in the arid zones (semi-desert, coast) and in the physiologically dry habitats (serpentine, limestone karsts, white sands). Also, the majority of endemics have xeromorphic leaves. Table 1, and Fig. 3 show the

Table 1

The leaf-size distribution of 1115 tree and shrub species occurring in 40 native forest communities of Cuba

| Leaf-size category | Non endemics | | Endemics | | Total | |
|--------------------|--------------|-----|----------|----|-------|-----|
| | No. | % | No. | % | No. | % |
| Macrophyll | 6 | 100 | 0 | 0 | 6 | 100 |
| Mesophyll | 58 | 65 | 30 | 35 | 88 | 100 |
| Notophyll | 96 | 43 | 125 | 57 | 221 | 100 |
| Microphyll | 163 | 33 | 337 | 67 | 500 | 100 |
| Nanophyll | 45 | 22 | 155 | 80 | 200 | 100 |
| Leptophyll | 14 | 20 | 56 | 80 | 70 | 100 |
| Aphyll | 5 | 18 | 25 | 82 | 30 | 100 |

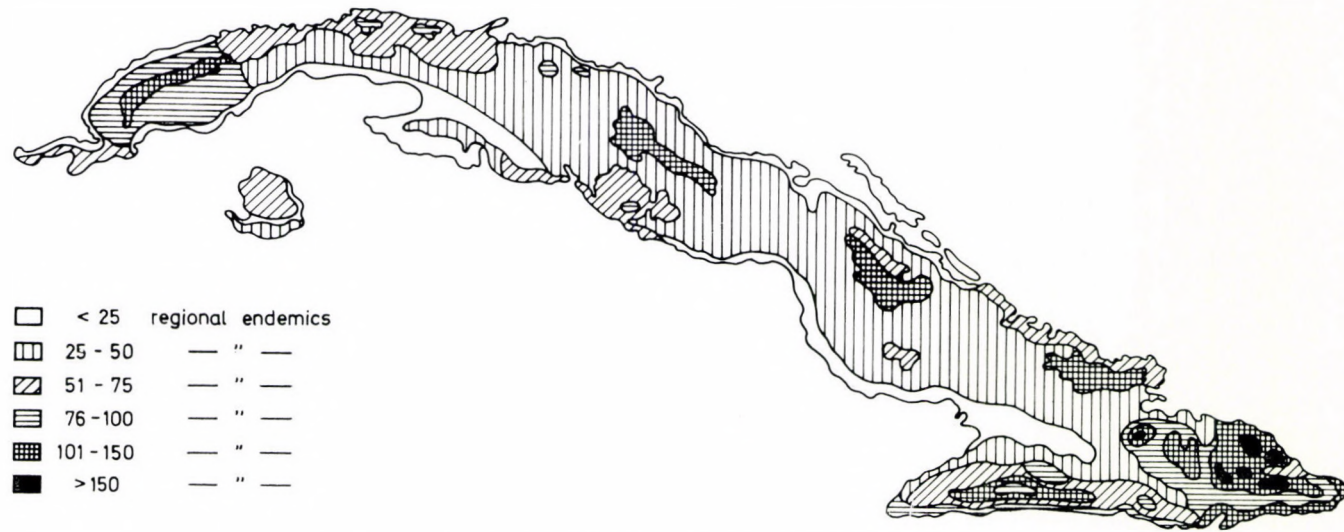


Fig. 1. Number of the regional endemic species per area

leaf-size distribution of 1115 tree and shrub species occurring in forty native forest communities of Cuba. Among macrophylls and mesophylls the widely distributed species dominate, but in the microphyll, nanophyll, leptophyll and aphyll categories the endemics outnumber the others and their proportion increases as leaf-size decreases.

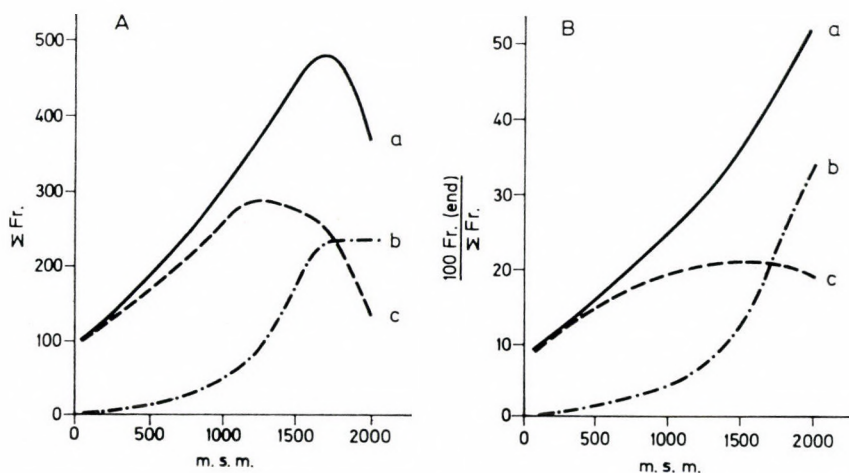


Fig. 2. Vertical distribution pattern of endemic species in Cuba (A), and their relative frequency in the zonal vegetation types (B) along a vertical gradient. — a: total of endemics, b: local montane endemics, c: regional lowland endemics

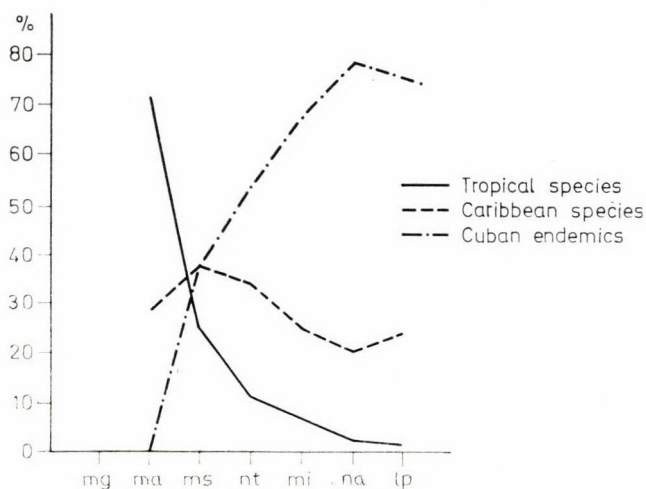


Fig. 3. The leaf-size class pattern of Cuban trees and shrubs — mg: megaphylls, ma: macrophylls, ms: mesophylls, nt: notophylls, mi: microphylls, na: nanophylls, lp: leptophylls and aphylls

Disjunction

Another chorological characteristic of the flora is disjunction, the separation of the geographic range of a given taxon into several isolated areas. Many different types of disjunction, such as bipolarity, bi-, tri-, and multi-sectorial distribution may be observed in Cuba.

Bipolarity

The most striking type of disjunction is the bipolarity of the geographical range of certain genera (SAMEK 1973) that occur only in western Cuba and Isla de Pinos, and also in the mountainous regions of Oriente. This is the case with *Purdiaea* (Fig. 4), *Heptanthus* (Fig. 5), *Spathelia* (Fig. 6), *Pinus*, *Podocarpus* and many other genera. The reason is that the eastern and western parts of Cuba has been isolated for a long period of time from the beginning of the Tertiary. In most cases, the number of species in the disjunct genera is higher in Oriente than in western Cuba. The larger area and more diversified landscape of Oriente cannot explain this observation, since the Sierra Maestra emerged only at the end of the Tertiary, and western Cuba has more diverse geological conditions than Oriente. A more acceptable interpretation is that Oriente and the continent had been connected through Hispaniola for a relatively longer period of time, facilitating a steadier "gene supply" for this province.

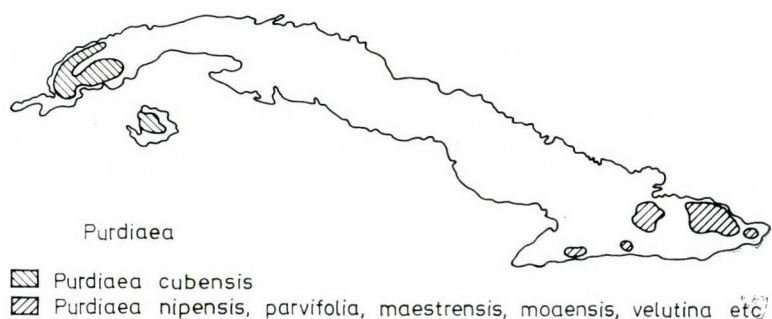


Fig. 4. The geographical distribution of the genus *Purdiaea* in Cuba (after THOMAS 1960, SAMEK 1973 and BORHIDI 1973)

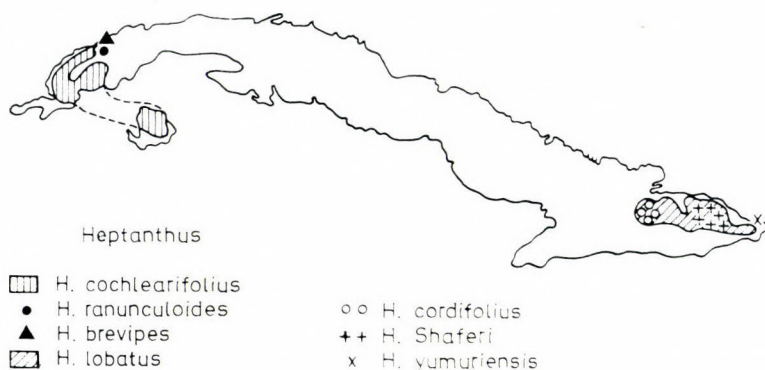


Fig. 5. The geographical distribution of the genus *Heptanthus* Griseb. (after BORHIDI 1972)

Geologists (ARNDT 1917–1922; SCHUCHERT 1935) pointed out that the separation of western and eastern Cuba preceded that of Oriente and Hispaniola, although botanists, following URBAN (1923 p. 52), disagreed. The present analysis of the flora, however, provides a botanical evidence for the geological results. It is noted that G. SILVA (1979) arrived at the same conclusion in his studies on the Chiroptera fauna of Cuba.

At species level only a few examples of bipolar distribution exist. These species are found mainly on serpentines, e.g., *Amyris lineata*, *Vernonia angustissima*, *Croton bispinosus*, and *Helicteres trapezifolia*, but also in limestone karsts, such as *Byttneria microphylla* and *Neoregnellia cubensis*.

Bisectorial geographic distributions

Another type of disjunction is represented by some rainforest species (*Magnolia cubensis*, *Hedyosmum grisebachii*, *Ocotea ekmanii*, see Fig. 7) occurring both in the mountains of Oriente and the Guamuhaya massif. Up to the early Tertiary, these regions were connected as still indicated by the presence of the relict block-mountains of Sierra de Najasa. This con-

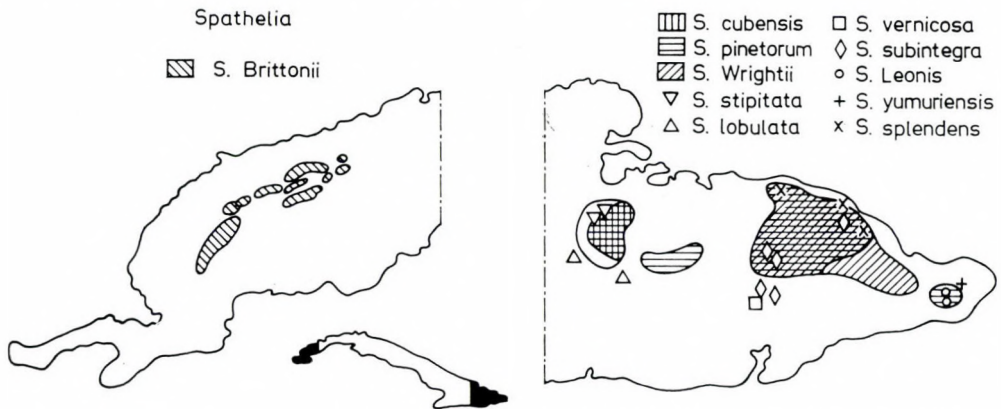


Fig. 6. The geographical distribution of the genus *Spathelia* in Cuba (after MARIE-VICTORIN 1944, SAMEK 1973, complemented by BORHIDI)

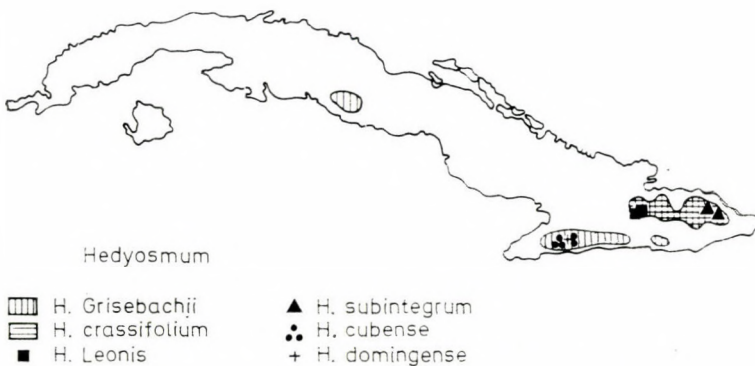


Fig. 7. Geographical distribution of the *Hedyosmum* species in Cuba (after MARIE-VICTORIN 1942, modified)

nection, however, must have come to an end in short time, since most common elements belong to ancient dicotyledonous families (Magnoliaceae, Lauraceae, Chloranthaceae, etc.). Other two types of the bisectorial distribution have developed between the mountainous region of western Cuba and the Guamuhaia massif:

1. the plants of karstic forests and other limestone areas (*Sapium leucogynum* and *Miconia cubensis*), and
2. species on serpentines in Pinar del Rio and the Escambray mountains (*Coccothrinax crinita* and *Linodendron venosum*).

Trisectorial distributions

The occurrence of several taxa in three isolated spots (western Cuba, Guamuhaia massif, Oriente) indicates that these areas had been separated for a long period of time in the Tertiary as a result of shallow-sea transgression of the island. The genera of *Lyonia* and *Vaccinium*; some montane forest species, such as *Tetrazygiopsis laxiflora* and *Sapium pallens*; and some acidophilous species, e.g., *Hypericum stypheloides* exhibit such a trisectorial distribution. As a consequence of longer geographic and ecological isolation of the *Hypericum stypheloides* populations, this species falls into three subspecies (LIPPOLD 1971). Some taxa considered earlier to have bi- or tri-sectorial distribution, proved to be taxonomically heterogeneous, such as *Amyris lineata*, *Croton brittonianus*, *Ditta myricoides*, *Lyonia elliptica*, and *Vaccinium cubense*.

Multisectorial areas

Several serpentine species (Figs 8–9) have relatively large geographic range falling into many isolated spots. In these cases the reason of disjunction is the scattered occurrence of acceptable habitats, rather than the prehistoric geographic effects. Examples are *Phyllanthus orbicularis*, *Neobraccia valenzuelana*, *Rondeletia camarioca*, and *Jacaranda cowellii*. This type of geographical range is characteristic of the common species of the numerous isolated serpentine areas, as well as the plants of the isolated coastal limestones (*Castela calcicola*, *Machaonia havanensis*, *Dendrocereus nudiflorus*, and *Neobraccia angustifolia*).

Vicariancy

The third characteristic feature of the flora of Cuba is the abundance of vicarious taxa. All types of vicariancy are widespread. This fact indicates prehistoric isolations and allows the surveyor to make inference concerning the influence of ecological factors on evolution and the development of certain related taxa.

Geographic vicariancy

Many examples exist in Cuba. Figure 10 shows the range of two endemic vicarious genera of lianes, *Lescaillea* and *Harnackia*. Both occur in pinewoods and evergreen shrublands on latosols derived from serpentine. This serves as an evidence suggesting a relationship not only between the recent vegetation of the Cajalbana and Nipe Mountains, but also in the past flora development of these regions. Further examples are *Anemia coriacea* (Fig. 11) and *Moacrotroton* (Fig. 12), among many others. The ranges of *Thrinax* and *Hemithrinax* also exemplify geographic vicariancy (Fig. 13). *Thrinax radiata* is a species of rocky and sandy beaches,

whereas *Th. morrisii* and *Th. drudei* occur in the karsts of western Cuba replacing each other. In central and eastern Cuba this genus is substituted by *Hemithrinax* (*H. ekmaniana* and *H. compacta*). The ecological vicariads of them, namely *H. rivularis* and *H. savannarum*, occur on the serpentines of Moa. The latter two are cenological vicariads of each other, being forest and scrub species, respectively.

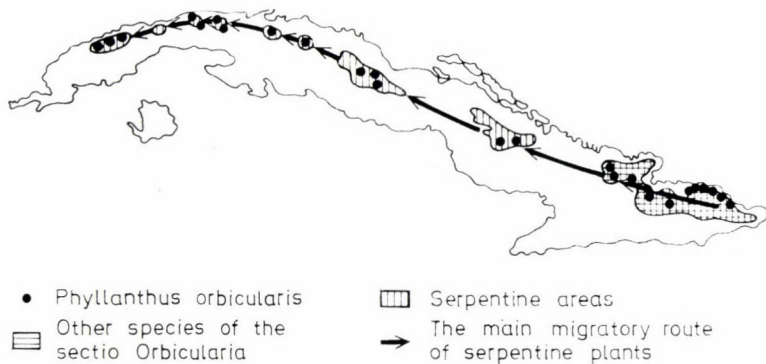


Fig. 8. Geographical distribution of the serpentine areas and the *Orbicularia* section of the genus *Phyllanthus* in Cuba (after WEBSTER 1958)

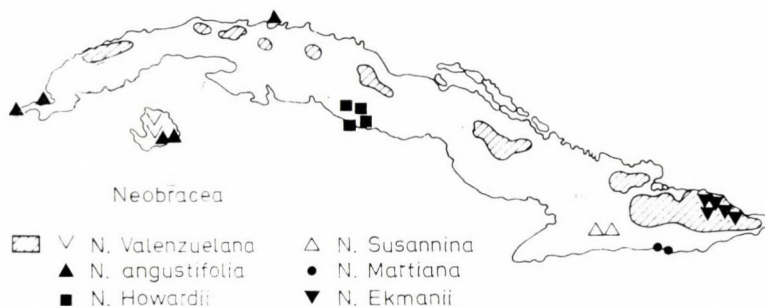


Fig. 9. The geographical distribution of the genus *Neobræcia* Britt. in Cuba (after BORHIDI 1973 and LIPPOLD 1979)

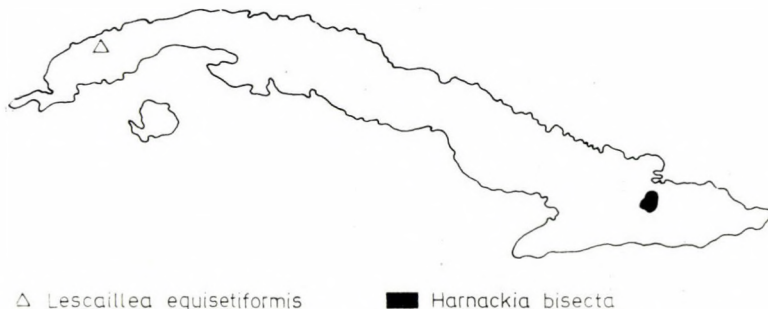


Fig. 10. The geographical distribution of the genera *Lescaillea* Wr. in Sauv. and *Harnackia* Urb. (after BORHIDI 1973)



Fig. 11. Geographical distribution of *Anemia coriacea* Griseb. s.l. (after BORHIDI 1973 and DUEK 1975)

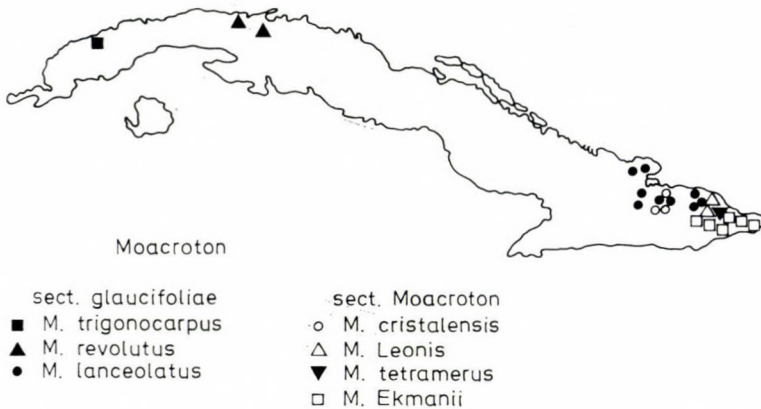


Fig. 12. Geographical distribution of the genus *Moacroton* Croiz. (after BORHIDI 1973)

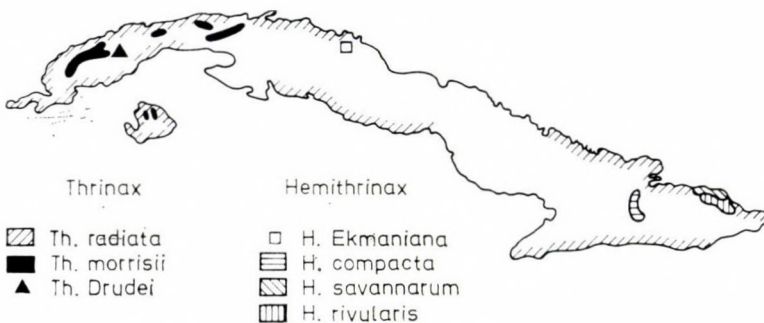


Fig. 13. Geographical distribution of the genera *Thrinax* and *Hemithrinax* in Cuba (after BORHIDI 1973 and READ 1976)

Combinations of geographic and ecological vicariancy

The genus *Platygyne* (Fig. 14) exhibits both types of vicariancy discussed so far. *P. hexandra*, an ubiquitous species found all over the country, is replaced by *P. parviflora* on serpentines in Camagüey and Holguín, by *P. dentata* in the montane zone of Sierra Maestra, and by other four species in the serpentine mountains of northeastern Oriente. Furthermore, the latter four species are also vicarious in their geographic distribution within the province.

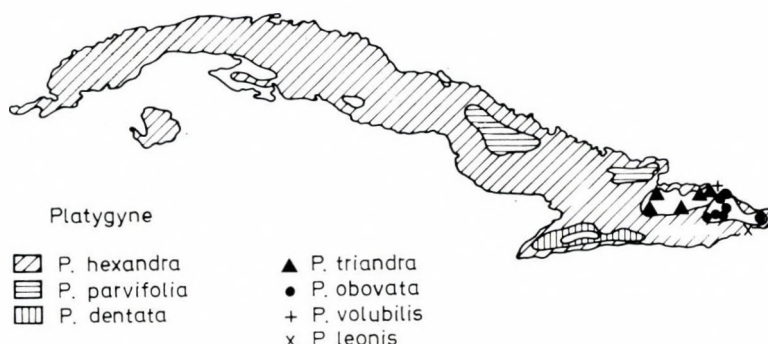


Fig. 14. Geographical distribution of the genus *Platygyne* Muell. Arg. (after BORHIDI 1972)

This observation supports the view that speciation is more intensive on serpentines than on other bedrocks (BORHIDI 1972a). An interesting exception is the genus *Neobraccia* (Fig. 9) with two species on serpentine, *N. ekmanii* endemic to Moa and the Pan-Cuban *N. valenzuelana*. Although the latter occurs on all serpentine outcrops in Cuba, the morphological variability of populations living under different climatic conditions remains within specific limits. Conversely, the ancestral form of the limestone species has been broken into four vicarious species along the coasts consisting of old and recent limestones. These species constitute morphologically well-separated small populations with considerably isolated geographic distributions.

Ecological vicariancy at the infraspecific level

The ecological factors affecting speciation may be best investigated and demonstrated through examples of infraspecific vicariancy. This term means that a young species of great migratory and penetrative power colonizes new habitats which in turn modify the populations. *Maytenus buxifolia* (Fig. 15), an Antillean species, is a good example. It falls into five vicarious subspecies in Cuba: the widely spread ancestor, two subspecies on serpentines (ssp. *cajalbanica* in western Cuba and ssp. *serpentini* in the east), another adapted to montane belt (ssp. *monticola*), and an extremely drought-tolerant subspecies in the semi-desert belt (ssp. *cochlearifolia*). An endemic microphyllous drought-tolerant shrub, *Reynosia mucronata* (Fig. 16) exhibits similar vicariancy. The ancestor occurs in the shrublands of central Cuba with preference in favour of neither limestone nor serpentine. However, the populations on the latosols of Nipe Mts. (ssp. *nipensis*) and the stands occurring in the semi-desert zone of southern Baracoa (ssp. *azulensis*) have been geographically isolated subspecies (cf. BORHIDI and MUÑIZ 1971).



Fig. 15. The geographical distribution of the subspecies of *Maytenus buxifolia* (A. Rich.) Griseb. in Cuba (after BORHIDI 1973)

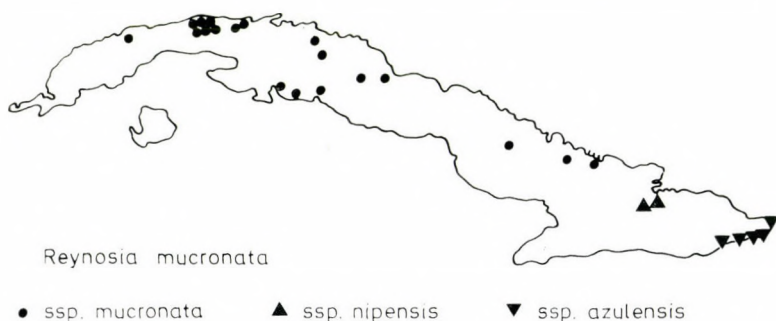


Fig. 16. The geographical distribution of the subspecies of *Reynosia mucronata* Griseb. in Cuba (after BORHIDI 1973)

Geographic vicariance at the infraspecific level

The infraspecific geographic vicariance indicates climatic changes in the recent geological past and casts light upon the role of potential cenological barriers. Examples are found in the serpentine mountains of northeastern Oriente. The climate of this region might have been that of tropical rainforests in the Holocene. Since then, a gradually drying climatic gradient has developed. As a result of this change some species, e.g., *Casasia nigrescens* (Fig. 17), *Amyris stromatophylla* (BORHIDI and MUÑIZ 1973), *Manilkasa mayasensis* and *Anemia coriacea* (Fig. 11) fall into vicarious subspecies with large leaves and tall habit in Moa and with small leaves and body size in the Cristal and Nipe Mountains.

Phytosociological isolation and vicariance

Development of geographically vicarious subspecies may often be facilitated by the existence of species-saturated communities or vegetation types in different habitats separating the drifted populations, e.g., those of mountains alternating with more or less wide valleys. Here, only the distribution of the taxa of *Calycogonium rosmarinifolium* s. l. (Fig. 18) is shown, although the vicarious subspecies of *Phyllanthus erythrinus* (WEBSTER 1959) could have also been selected as a good example.

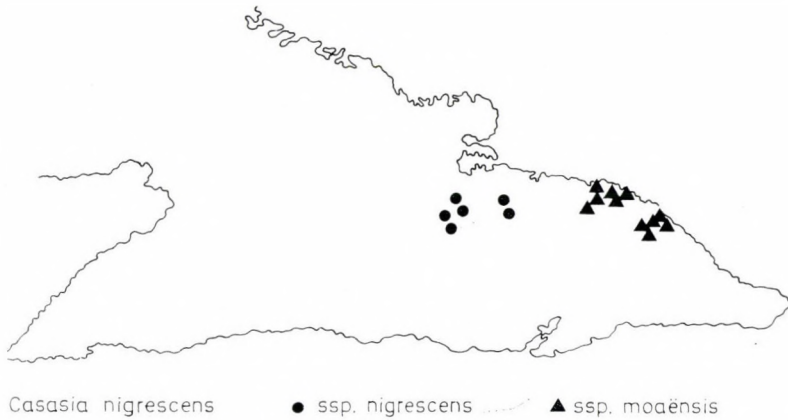


Fig. 17. The geographical distribution of the subspecies of *Casasia nigrescens* (Griseb.) Wr. ex Urb. in Cuba (after BORHIDI 1973)

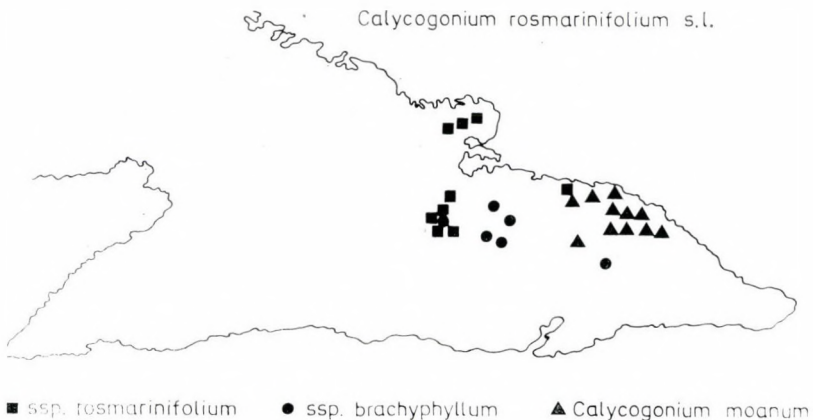


Fig. 18. The geographical distribution of the taxa of *Calycogonium rosmarinifolium* Griseb. s.l. (after BORHIDI 1973)

Inversion of floristic elements

Another typical feature of the flora, observed mainly in western Cuba, is inversion (SAMEK 1973). Certain genera of mountains or temperate regions, which otherwise occur positively in high mountains at similar latitude, are found in Cuba at low elevation. Examples are *Pinus*, *Podocarpus*, *Kalmiella*, *Vaccinium*, *Lyonia*, *Befaria cubensis*, and *Quercus sagraeana*, etc. Inversion is probably caused by two factors:

1. One of the cool periods during the Quaternary when these species or their ancestors reached Cuba and became established in the lowlands and hills, and

2. The abundance of serpentines and other rocks and soils (e.g., slate and quartz sand) of poor nutrient supply, which might have facilitated the adaptation of cool-resistant species to the increasing mean temperature (such inversions may develop under the same conditions even today).

The vegetation inversion occurring on soils derived from serpentine is discussed in BORHIDI (1973, 1976) and BORHIDI and MUÑIZ (1980). Of course, this is accompanied with the inversion of floristic elements constituting the corresponding vegetation type. In addition to the above mentioned taxa, this phenomenon is exemplified by the species of *Myrica*, *Ilex*, *Lobelia*, *Laplacea*, and *Baccharis* occurring on serpentines or white sands.

Microphyllia

This is an important feature characteristic not only of the flora of Cuba but also of the flora of the West Indies. As pointed out in earlier, aridity must have had an important role in influencing speciation. Table 1 shows that of the 1115 species examined, 800 (72%) proved to be microphyll, nanophyll, leptophyll and aphyll, most of those (563 species) being endemics. There are numerous genera represented in Cuba and the Antilles by highly specialized microphyll, sclerophyll or spiny sections or species, but the corresponding taxa in the continent are broad leaved. Examples are *Plinia*, *Myrcia*, *Calyptanthus*, *Eugenia*, *Rondeletia*, *Machaonia*, *Psychotria*, *Phyllanthus*, *Croton*, *Acalypha*, *Jacaranda*, *Tabebuia*, *Byrsonima*, *Malpighia*, etc. Typical West Indian genera are *Catesbaea*, *Scolosanthus*, *Ottoschmidtia*, *Acidocroton*, *Picrodendron*, *Krugiodendron*, and *Sarcomphalus*. Their abundance and substantial cover in the vegetation allow for the conclusion that during the main period of the flora development in the West Indies there was a direct relationship to the continental flora composed of drought-tolerant elements. Then, the flora must have further evolved in a warm, arid subtropical climate, probably in the second half of the Miocene.

Micranthia

A striking feature of the flora of the Antilles, and particularly of Cuba, is its richness in small-flowered plants, whereas the floras of Palaeotropical and Neotropical regions in the continents abound in showy, big-flowered species.

Among many thousands of plant species illustrated in Botanical Magazine and Garden-flora, Antillean species only occasionally occur. (None of the 3000 endemics in the exceptionally rich flora of Cuba has big enough flowers to deserve the honour of being the 'national flower', so *Hedychium coronarium*, an Asiatic species, was given this title.)

The small flowers are pollinated by endemic, highly specialized microscopic sized insects that are usually not capable of long distance flying. To avoid strong winds blowing from the sea, these insects stay in the shelter of plants. Thus, gene flow between remote plant populations is occasional, if not impossible. This barrier must have contributed significantly to the isolation of populations subjected to genetic drift, and resulted in the formation of numerous vicarious endemics. The fact that most vicariads differ very little from one another indicates the influence of genetic drift. Therefore, some taxonomists consider these vicariads as simple varieties. However, this view is unacceptable since the morphology of these microspecies is stable, the segregation is complete and no intermediate forms and genetic relationships exist. *Micranthia* may be a partial explanation of the discrepancy that genera represented by only one or two widespread species in the continent may fall into a dozen of endemic species with geographic range restricted to a single mountainous region in the Antilles.

Relict character

The flora of tropical moist vegetation types, such as lowland and submontane rainforests and seasonal evergreen forests in the Antilles is relatively young and contains few endemics. It is likely that these types had become widespread only at the end of the Pliocene and during the pluvial periods synchronous with the Pleistocene glacials. During the Pleistocene the flora rich in Tertiary xerophilous and sclerophyll elements retreated. This process still goes on. In my opinion the recent climate is far too moist for the sclerophyll vegetation types and the constituting species. The increase in the number of permanently established deciduous and semi-deciduous species coming from other community types supports this view. This degradation process is significantly accelerated by human impact. The formerly widespread dry evergreen formations (thickets, woodlands, forests and pinewoods) composed of Tertiary sclerophylls are now restricted to relict habitats, serpentines and the slopes and cliffs of conical karsts. The geographic range of endemic sclerophylls, which account for 40% of the flora, covers less than 20% of the area of the island. The relict character of the flora is clearly demonstrated by the presence of primitive, taxonomically isolated groups (*Microcycas*, *Dracaena*, *Cneorum*, *Spathelia* sect. *Brittonii* and sect. *Splendentes*, and *Harpalyce* sect. *Cubenses*, etc.), the abundance of disjunct geographical distribution types, and the large number of local endemics represented by small populations.

Vulnerability

In general, island floras are vulnerable for several reasons:

1. The populations colonizing new biotopes are selected from a reduced gene pool,

2. There has been no possibility of changing this gene pool for millions of years,

3. As a consequence of isolation, the ecological tolerance and genetic flexibility of populations decreased, so that,

4. The competitiveness of species generally diminished or even disappeared in all but one respects,

5. Therefore, they cannot react satisfactorily upon new ecological impacts, cannot take the advantage of succession, and cannot resist to or force back new competitors.

Thus, island floras are usually composed of ecologically rigid populations not qualified to adapt to major environmental changes. The social recuperability is reduced, the populations and communities are vulnerable to external effects. The vulnerability of the flora of Cuba is more pronounced because

a) The island flora itself is constituted by set of ancient, isolated floras,

b) Most endemics have been adapted to the extreme ecological conditions of oligotrophic or bare areas. Thus, the level of metabolism became low in these organisms, and their competitiveness diminished.

c) The sociability of many endemics is low. There are some rare taxa represented by few populations, and few individuals therein. Consequently, the flora of Cuba is one of the most endangered island floras of the world. The list of endangered species of Cuba contains about a thousand items which amount to 16.5% of the entire set of flowering species in the island. Approximately 30% of the endemics are endangered.

The origin and migration of the flora of Cuba

Palaeobotanical and geological evidences

Our knowledge on the origin and migration of the flora of Cuba is insufficient. As few as three noteworthy findings have been reported, one probably from the Eocene and another from the Pleistocene of Sierra de Chorrillo, and the third from the Miocene of Yumuri Valley in Matanzas (see GRAHAM 1973). The total number of taxa coming from these three periods is still below fifty. Moreover, no palynological data are available from the Quaternary of Cuba. Then, the only possibility to give a rough picture on past changes in the flora is offered by the simultaneous analysis of the recent flora, geographical distribution types and of the tectonic events revealed by structural geology.



Fig. 19. The geographical position of the Greater Antilles in the late Miocene according to WEGENER's continental drift theory in the interpretation of CORRAL (1939)

CORRAL's version of WEGENER's theory

The geological past of the Antilles has been described by CORRAL (1939, 1940) in accordance with WEGENER's theory (Fig. 19). It is conceivable that portions of the Greater Antilles used to be connected to one another and to South America, as CORRAL claims. However, these lands must have started to become separated from the continent not later than the Upper Triassic. Due to chronological unreliabilities and consequential inconsistencies in CORRAL's theory, it was soon rejected (ALAIN 1958) and SCHUCHERT's (1935) concepts were accepted.

SCHUCHERT's concepts on the Tertiary in the Antilles

According to SCHUCHERT, the Greater Antilles had been connected to Central America via a land strip of Honduras from the Triassic to the Late Pliocene, apart from short intervals in the Upper Cretaceous and the Upper Oligocene. Eastern Cuba was connected to the continent via the Cayman Ridge and indirectly via the Nicaragua Ridge, Jamaica and Hispaniola. In addition, there was also a connection to the Bahamas and Florida during the Upper Cretaceous and the Eocene. In the Upper Oligocene Cuba became separated from Hispaniola and Yucatan, and its connection to the continent ceased to exist any longer. Although in the Middle Miocene Cuba and Hispaniola had been united again for a short time, no link was formed between the Antilles and Honduras. In the Upper Miocene Cuba finally became completely separated from all the other islands of the Antilles and the continent. According to SCHUCHERT, the Honduras-Jamaica-Hispaniola land strip was finally broken only at the beginning of the Pleistocene. In my views, however, this break up must have been completed in the Miocene, as suggested by the very primitive mammal fauna of the Greater Antilles.

The influence of SCHUCHERT's concepts on phytogeographical interpretations

The recent position of the West Indies, which are wedged in between two continents and are surrounded by narrows and closed bays, makes the impression as if the West Indies were a sort of appendage of the continent that became separated not long before. Some botanists consider the flora of the West Indies as a descendant of the continental flora in which the widespread and highly variable continental species are represented by endemic subspecies

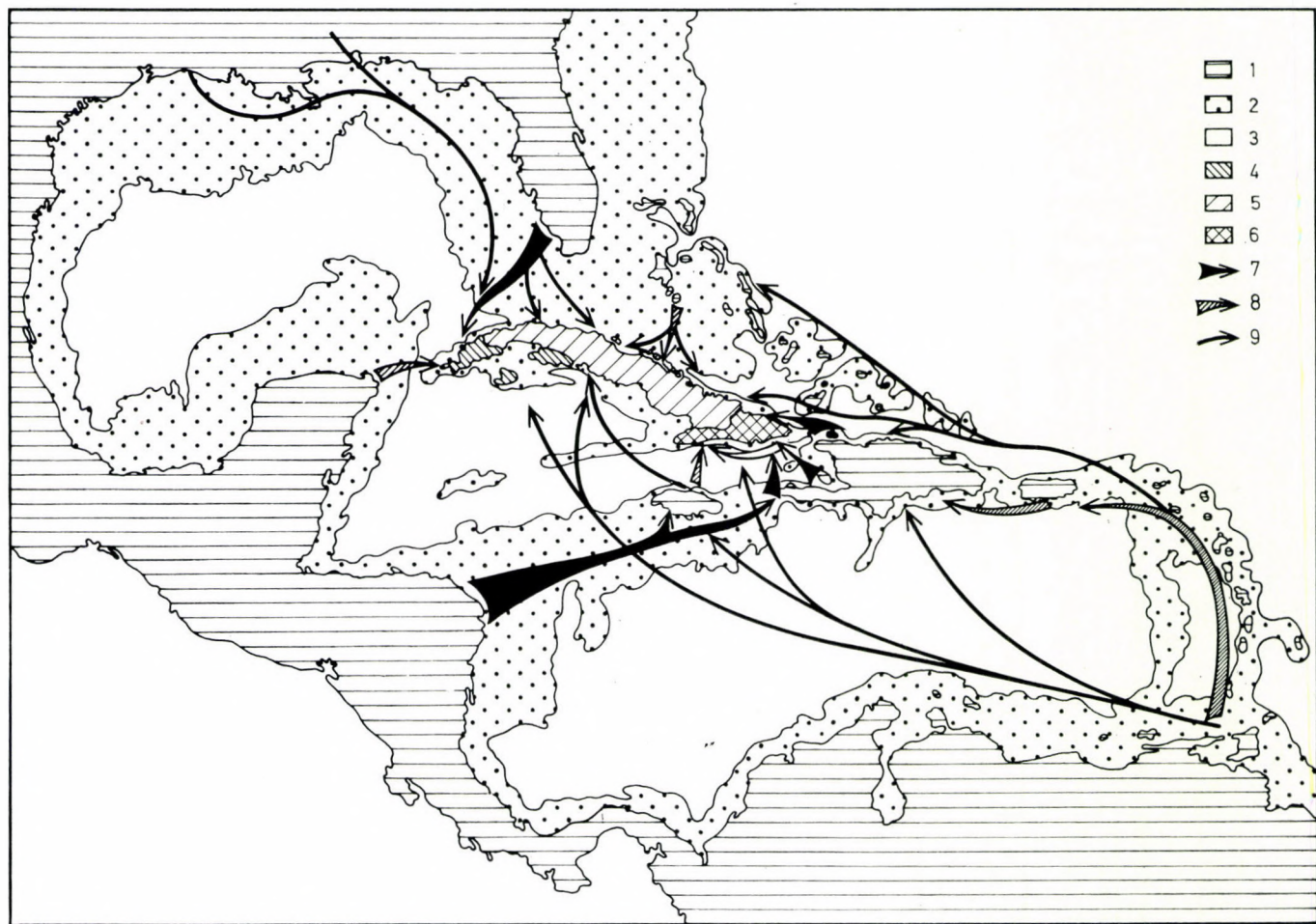


Fig. 20. Origins of the flora of Cuba (after ALAIN LIOGIER 1958, strongly modified). 1. Continents and islands, 2. Shallow seas (less than 1000 m deep), 3. Deep seas (depth more than 1000 m), 4. West-Cuban phytogeographical subprovince, 5. Middle-Cuban phytogeographical subprovince, 6. East-Cuban phytogeographical subprovince, 7. Main migratory routes of the flora, strong floristic affinities, 8. Important migratory routes, notable floristic relations, 9. Less important migratory routes of the flora, recognizable floristic relations

or varieties. This is, however, an illusory view formed under the influence of SCHUCHERT's theories discussed above. Given this knowledge, the first fundamental statements regarding the origin of the flora of Cuba were made by ALAIN (1958 pp. 33-34). He suggests that the flora is mainly of Neotropical origin and the elements immigrated from five different directions (Fig. 20). This problem will be considered later in more detail.

The influence of plate tectonics

The results of contemporary plate tectonics gave an impetus to the research of flora evolution. Several new hypotheses were created concerning the evolution and migration of primitive floras and the radiation of Gondwana elements, etc. At the same time, controversies among experts also revived. Several influential biologists, e.g., THORNE (1973) and VAN STEENIS (1962) in botany, maintain their conservative view. They claim that the division and drift of continents need not be assumed to understand the recent state of floras. As VAN STEENIS asserts, for example, the Pacific and the Indonesian flora may have been directly originated from Asia via land bridges that have now been covered by sea. Even if the land bridge theory is applicable to the Indonesian flora, there are still many paleogeographic and biogeographic problems that cannot be solved in this way. It is therefore obvious that most botanists call in the theoretical possibilities offered by plate tectonics, even though this field of geology has its own unanswered questions.

Plate tectonics of the West Indies

Despite many controversial details, most geologists agree that the Caribbean region is a separate tectonic unit. After ISACHS et al. (1968) and MORGAN (1958) the existence of a separate Caribbean Lithosphere Plate is generally accepted. As far as the origin and the characteristics of this plate are concerned, the views are varying. SCHUCHERT (1935), WOODRING (1954), EARDLEY (1954), BUTTERLIN (1956), TIKHOMIROV (1967), and KHUDOLEY and FURAZOLA (1971) think that the Caribbean Sea took the place of a land mass. H. A. MEYERHOFF (1954), HESS and MAXWELL (1953), WEYL (1966), A. A. MEYERHOFF (1967), DENGÖ (1968), MOLNÁR and SYKES (1969), and MACGILLAVRY (1970) presume the existence of a permanent basin in the ocean surrounded by island bends and geosynclinals.

Most experts (BALL and HARRISON 1969, DENGÖ 1969, MOLNÁR and SYKES 1969, MACGILLAVRY 1970, MALFAIT and DUNKELMAN 1972, MATTSO 1973, and ITURRALDE 1975, 1978) explain the development of the Caribbean Plate on the basis of continental drift and, in general, of the dilatation of ocean floor.

The unanimous view of the above authors is that the Caribbean Plate was primarily oceanic. They exclude the possibility that the Antilles were connected to either part of the American continent up to the Middle Miocene. In the Upper Jurassic the Caribbean Plate may have been located at the northwestern edge of the Afro-American land mass in the Pacific Ocean at the western mouth of the Tethys Sea (where Nicaragua and Honduras are now found). In the Cretaceous, simultaneously with the process separating South America and Africa, the South American continent was significantly rotated causing the Caribbean Plate, which was connected to the Guyana Plate, to move eastward. Having moved about 800-1200 kilometres, the Caribbean Plate drifted away into the Tethys Sea. As from the Upper Miocene the Antilles exhibited a platform-like development. According to ITURRALDE, Cuba has been formed of six archaic isolated blocks, Hispaniola probably of three blocks, and Jamaica and Puerto Rico of one block each. In the Upper Miocene started the general vertical

movement leading to the emergence of the Cayman and Nicaragua Ridge. Thus, the archipelago and Central America became connected. This process was completed in the Pliocene and then the islands started to get separated. Finally, the recent shape of the islands was formed in the Pleistocene.

The theory of a three-phase evolution of the West Indian flora

Although Caribbean Plate tectonics poses many unresolved problems (HOWARD 1980 p. 244) it is now attempted to outline the general history of the Antillean flora. Three major periods are recognized, each corresponding to a given geological stage:

1. Plate phase,
2. Land bridge phase, and
3. Archipelago phase.

Plate phase

The name of the first stage indicates that at this time the Caribbean Plate was an independent land mass in the sea. It is assumed that this period started in the Middle Jurassic and ended in the Upper Oligocene. Relatively few elements of the flora may be traced back with complete certainty to this phase. These important taxa are either phylogenetically old relicts, or endemics with distant relations, or genera with disjunct geographic distribution.

Relict cryptogams

As expected, the flora of this early period is mainly represented by cryptogams in the recent flora. Such plants are, for instance, the tree ferns. As many as seventeen endemic tree ferns occur in the Antilles. It is well-known that the family Cyatheaceae includes widely distributed species that are efficiently dispersed through spores. The fact that one of the evolutionary centres of the Schizaeaceae family, and its genus *Anemia*, is in Cuba (12 species of which seven are endemics) needs similar interpretation. In this respect, new bryogeographic results (REYES 1982, BORHIDI et PÓCS 1985) deserve particular attention as they indicate a definite relationship to Gondwanaland. For instance, there are many more liverwort species than mosses in Cuba. Such a partition of bryophytes is known to be typical of the islands in the southern hemisphere. The new studies by PÓCS and REYES revealed obvious relationships between the liverwort floras of the Guyana Highland and of the serpentine plateaux in Cuba, e.g. by the occurrences of *Plagiochila steyermarkii* Robinson and *Calypogeia venezuelana* Fulford. It is well-known that the Guyana Highland is one of the oldest block mountains in South America derived undoubtedly from Gondwanaland.

Relict phanerogams

The monotypical gymnosperm genus *Microcycas* is a "living fossil" representing the phanerogams of the plate phase. The assumption that at that time the Caribbean Plate was located at the border of the Pacific Ocean and the Tethys Sea has not only been justified by fossil Cretaceous faunas (western Cuba, Viñales) but has also been demonstrated by the close relationship between *Colpothrinax*, a Cuban-Central American genus, and the Pacific *Pritchardia*. These genera probably have a common ancestor.

Peri-Afro-American elements

The evolution of the specially distributed peri-Afro-American elements may also be dated back to the plate phase. Table 2 lists typical peri-Afro-American phanerogam genera that occur in the tropical America, East Africa and/or Madagascar but are absent from West and Central Africa.

Of the fifteen genera listed, eleven occur in Cuba, four genera (*Carpodiptera*, *Savia*, *Oplonia*, and *Stenandrium*) have their evolutionary centres in the Antilles (BORHIDI 1982).

According to STEARN (1971) this distribution type may have been developed as a result of drastic climatic changes. Many taxa became extinct in the interior of the "Afro-American" supercontinent due to the extreme continental climate. The flora of Central Africa became relatively poor in species. On the contrary, along the coasts of Madagascar, East Africa, North Africa and the northern part of South America an extensive coastal zone was formed which had a temperate climate. The abundance of diverse habitats in this region was advantageous for survival so that a very rich vegetation developed. Besides the Madagascar Plate, the Macaronesian and Caribbean Plates were probably also parts of this coastal zone.

In addition to the genera mentioned so far, a similarly close relationship is indicated for *Dracaena cubensis* from Cuba, *D. draco* from Macaronesia and *D. reflexa* from Madagascar. The primitive Cnecoreaceae family may also be mentioned as a good example (BORHIDI 1982). It has only three living species, one in the Mediterranean of Europe, another in the Canary Islands and the third in eastern Cuba on the southern slopes of Sierra Maestra. Further evidence confirming the existence of these relationships is that amongst the cryptogams there are not only genera but also some species exhibiting peri-Afro-American distribution (e.g., liverworts).

Table 2*Examples of peri-Afro-American genera*

| Genus, Family | Total species | S. America, W. Indies | W. and Central Africa | E. Africa, Madagascar |
|----------------------------------|---------------|-----------------------|-----------------------|-----------------------|
| <i>Desmanthus</i> (Fabaceae) | 22 | 15 | 1 | 6 |
| <i>Echinochlaena</i> (Poaceae) | 7 | 6 | — | 1 |
| <i>Ocotea</i> (Lauraceae) | 400 | 380 | 3 | 18 |
| <i>Oliganthes</i> (Asteraceae) | 21 | 12 | — | 9 |
| <i>Carpodiptera</i> (Tiliaceae) | 6 | 5 | — | 1 |
| <i>Oplonia</i> (Acanthaceae) | 18 | 13 | — | 5 |
| <i>Phenax</i> (Urticaceae) | 28 | 25 | — | 3 |
| <i>Piriqueta</i> (Turneraceae) | 28 | 24 | 1 | 3 |
| <i>Rheedia</i> (Clusiaceae) | 50 | 37 | — | 13 |
| <i>Ravenala</i> (Musaceae) | 2 | 1 | — | 1 |
| <i>Savia</i> (Euphorbiaceae) | 25 | 15 | 1 | 9 |
| <i>Stenandrium</i> (Acanthaceae) | 25 | 22 | — | 3 |

It is assumed that some primitive angiosperms have also been present in Cuba since the plate phase. These are genera with bi- or trisectorial distribution, e.g., *Podocarpus*, *Magnolia*, *Talauma*, *Ocotea*, *Persea*, *Guatteria*, *Oxandra*, *Hedyosmum*, *Dorstenia*, *Bonnetia*, *Laplacea*, *Freziera*, *Lagetta*, *Linodendron*, *Myrcia*, *Spathelia*, *Purdiaea*, and *Calophyllum* and those listed in Table 2.

Land bridge phase

The second period which lasted from the end of the Oligocene up to the end of the Pliocene may be termed the land bridge phase. At the beginning of the Oligocene started the Caribbean Plate to emerge. As a result of this process the Greater Antilles became connected to the continent first via Honduras and the Yucatan peninsula and later via the newly emerged Lesser Antilles. This is the period of the large scale immigration of species into Cuba because apart from facilitating migration, the emerged lands offered extensive bare areas for the pioneer vegetation.

The broad-leaved tropical Honduras flora

During the Oligocene and the Lower Miocene a tropical flora consisting primarily of broad-leaved evergreen trees and shrubs may have immigrated into the Greater Antilles. In this period Cuba had been divided up into six isolated blocks: 1. Guane, 2. Isla de Pinos, 3. Villa Clara, 4. Camagüey, 5. Sierra Maestra, and 6. Moa-Baracoa. In the Middle and Upper Oligocene Guane was connected to the Yucatan peninsula. The Sierra Maestra block and Honduras were connected via the Cayman Ridge. The Moa-Baracoa block joined probably with Hispaniola and formed a part of the Honduras-Jamaica-Hispaniola-Puerto Rico range which was the main route of immigration. Obviously, the relatively small Guane block, that became separated earlier from the continent, accumulated a more limited gene pool than the larger Oriente blocks (Sierra Maestra and Moa-Baracoa) which had been connected to the other parts of the Greater Antilles for a longer period of time. The plants of the latter blocks comprise the so-called Honduras flora which might also be termed the *Swietenia-Trichilia-Zanthoxylum* flora after its typical genera. Most Neotropical elements of the Greater Antilles have been originated from the Honduras flora. According to my hypotheses, it is the phase when three main groups of tropical genera immigrated. These are:

1. Genera represented by few but widely spread endemic species in the recent flora (*Swietenia*, *Trichilia*, *Cedrela*, *Inga*, *Albizzia*, *Bursera*, *Dipholis*, *Pseudolmedia*, *Chlorophora*, and *Simaruba*).

2. Genera with secondary evolutionary centres evolved due to climatic and geological changes in the Antilles. These genera have many endemic representatives in the recent flora (*Pithecellobium*, *Cassia*, *Caesalpinia*, *Copernicia*, *Eugenia*, *Calyptanthus*, *Zanthoxylum*, *Pilea*, *Rondeletia*, *Psychotria*, *Guettarda*, *Miconia*, *Ossaea*, *Phyllanthus*, etc.), and

3. Presumably extinct genera that are ancestors of typical endemic genera or genus groups characteristic of the Antilles, e.g., the group *Portlandia-Cubanola-Thogsennia*; the group *Schmidtottia-Isidorea*; the group *Phialanthus-Ceratopyxis-Shaferocharis*; the group *Antillia-Urbananthus-Spaniopappus*; the group *Scolosanthus-Eosanthus*; the group *Pinillosia-Tetraperone-Koehneola*; and the group of *Espadaea-Goetzea-Henoonia*, etc.

The sclerophyllous broad-leaved Madro-Tethyan vegetation

AXELROD (1975) recognizes that the high similarity between the mediterranean vegetation of North America and Eurasia-N. Africa is not merely a physiognomic resemblance because most genera occurring are common to both regions. He shows that a sclerophyllous broad-leaved vegetation developed in the Eocene and Oligocene of Sierra Madre (Mexico) and the southern United States, and also in the Oligocene and Miocene of southern Eurasia, with many species in common. This vegetation, quite uniform in appearance, was named

the Madro-Tethyan vegetation after its westernmost limit, Sierra Madre, and the Tethys Sea, along the northern coast of which this vegetation type was spread. It was widely spread, too in the Upper Miocene of Central Europe. For example, the Sarmatian flora described from Hungary (ANDREÁNSZKY 1956, 1962*a, b*) is also designated by AXELROD as being parts of the Madro-Tethyan vegetation. This sclerophyllous broad-leaved vegetation, which was formed by plants highly adapted to the increasing summer droughts in the Tertiary, has now been splitted into remote, isolated stands living under very different ecological conditions. In California and Europe the summer is dry and the winter is wet, in Arizona and New Mexico there are two dry seasons. This vegetation type occurs under the dry winter and rainy summer climate of Mexico and the West Indies. Moreover, extensive stands are also found in places where the climate is wet throughout the year, but there are some sufficiently dry or nutrient poor habitats thanks to edaphic effects (e.g., white sand, serpentine, granite-gneiss domes, lava, limestone dog-tooth areas, etc.).

The southern link

AXELROD pointed out correctly that the laurel forests of the Canary Islands belong to the Madro-Tethyan vegetation, representing its southernmost portion. However, he did not think of the sclerophyllous communities of the Antilles which are also a part of that section. In the Miocene Honduras, the Yucatan peninsula and the Greater Antilles, which were connected to the first two, formed the southern coast of the Tethys Sea in America. These areas were similar in effect to the northern coast of Africa and the Canary Islands on the other side of the Tethys Sea. Accordingly, a special type of the sclerophyllous Madro-Tethyan vegetation, which should preferably be termed the *Peri-Tethyan* sclerophyllous vegetation, developed in the Greater Antilles. In addition to the Madro-Tethyan elements, this Antillean type is characterized by its richness in Neotropical sclerophylls and particularly in endemics, and also by its isolation. No doubt that the serpentine charrascals (dry evergreen shrublands), pine-oak woodlands, pine-laurel forests, and pine-*Dracaena* forests of Cuba are relicts left of the southern portion of the Madro-Tethyan vegetation.

The Madro-Tethyan flora in Cuba

The sclerophyllous broad-leaved Madro-Tethyan flora immigrated into the Antilles presumably in the Lower and Middle Miocene. It was probably a direct continuation of the former invasion of the Honduras flora. In this period an increasingly arid subtropical climate was predominant not only in the tropical zone but also in most parts of the warm temperate zone. More recently, this unusual expansion of the subtropical zone is attributed to a temporarily formed ring around the Earth, similar to that of the Saturn. This dry period displayed a strong influence on the evolution of the Cuban flora, in which the sclerophylls are still dominant. 75% of the endemic species are micro- or nanophanerophytes (2265 species), most of them (86%) being micro-, nano- and leptophylls. Many genera typical of the Madro-Tethyan vegetation immigrated into Cuba and became important elements of the flora supposedly in this period. These genera are: *Pinus*, *Juniperus*, *Quercus*, *Juglans*, *Buxus*, *Ilex*, *Lyonia*, *Vaccinium*, *Pieris*, *Kalmiella*, *Berberis*, *Celtis*, *Prunus*, *Myrica*, *Acacia*, *Calliandra*, *Erythrina*, *Karwinskia*, *Zizyphus*, *Reynosia*, *Colubrina*, *Thouinia*, *Dodonaea*, *Myrtus*, *Psidium*, *Euphorbia*, *Amyris*, *Helietta*, *Auerodendron*, *Sarcomphalus*, *Rhacoma*, *Forestiera*, *Sabal*, *Coccothrinax*, *Maytenus*, *Paepalanthus*, *Lachnocaulon*, *Syngonanthus*, *Eriocaulon*, *Chaetolepis*, *Befaria*, and *Garrya*.

Neotropical sclerophylls in the Madro-Tethyan vegetation of Cuba

The southern part of the Madro-Tethyan vegetation has its own typical features. Numerous species of the earlier established tropical broadleaf Honduras flora became adapted to the new arid climate, and several secondary evolutionary centres developed. Different Neotropical families and genera, represented by membranous leaved macrophylls and mesophylls in the continent, suffered a change so that new species groups or entire sections containing only sclerophylls and coriaceous leaved microphylls appeared in Cuba. Such genera are, for example, *Croton*, *Phyllanthus*, *Harpalyce*, *Miconia*, *Ossaea*, *Plumeria*, *Jacquinia*, *Plinia*, *Eugenia*, *Diospyros*, *Antirhea*, *Exostema*, *Rondeletia*, *Guettarda*, *Machaonia*, *Melocactus*, and *Gesneria*, etc. The endemic genera of the Antilles, Hispaniola and Cuba, that are typical of the sclerophyllous vegetation types, may also be considered as results of this climatic change. Examples are *Acrosynanthus*, *Phialanthus*, *Neomazaea*, *Ariadne*, *Phyllacanthus*, *Sarcophalus*, *Doerpfeldia*, *Pachyanthus*, *Scolosanthus*, *Catesbaea*, *Notodon*, *Nashia*, *Sauvallella*, *Leucocroton*, *Phidiasia*, *Lescaillea*, *Harnackia*, *Phania*, *Shafera*, *Moacroton*, *Hemithrinax*, *Ceuthocarpus*, *Schmidtottia*, *Roigella*, *Suberanthus*, *Kodalyodendron*, *Henoonia*, *Linodendron*, *Adenaea*, and *Tetralix*.

Drought resistant ecotypes

The numerous newly occurred sclerophyllous species and genera were only one indication of the adaptation to the dry climate of the Miocene. The membranous leaved trees of the Honduras flora became also adapted to aridity by developing drought resistant ecotypes. Although there are very little morphological differences between these ecotypes and the corresponding Central American populations the habitats of these species are apparently drier in Cuba than in the continent. Examples are: *Ceiba pentandra*, *Trichilia glabra*, *T. hirta*, *Cupania macrophylla*, *Bursera simaruba*, etc.

Archipelago phase

The third period is termed the archipelago phase. It came about in different points of time over the Greater Antilles. In Cuba it started at the end of the Miocene, when the Bartlett Trench, that separates Cuba from Jamaica and Hispaniola, was formed. Of these islands Jamaica had been connected to the continent for the longest time, as clearly indicated by the floristic composition of this island (ASPREY and ROBBINS 1953, ADAMS 1972). Changes typical of this period are the ecological specialization of floras isolated from one another and, as a consequence of this, the internal migration of species. In this phase the flora and fauna of Cuba were subjected to severe climatic and geological changes. In the Pliocene and the Pleistocene the wet climate frequently alternated with dry periods. The effects of this climate are clearly indicated by the stratification of ferritic soils in the Nipe Mts. Cooler periods, too, were frequent in the Pleistocene. Large scale tectonic events took place simultaneously with the climatic changes. The most effective of them was that the six, formerly separate land blocks emerged and joined together to form a single island with extensive bare areas. The colonization of these lands required further differentiation and migration of the flora and the adaptation of species to the new environmental conditions. Other external effects, independently of the geological changes, also influenced the flora of Cuba.

The broad-leaved rainforest flora of Guyana

The direct land connections between Cuba and Central America disappeared by the end of the Miocene. Later, in the early Pliocene, the climate became wetter. In the second half of the Pliocene the arch of many young volcanoes, i.e., the Lesser Antilles, emerged and the islands of the Greater Antilles also reached the maximum elevation. Presumably, this is the period when the South American elements immigrated into Cuba via the Lesser Antilles, although at that time land strips no longer existed. Yet, the genera *Carapa*, *Ochroma*, *Coussarea*, *Tocoyena*, *Paratheria*, *Phinaea*, *Proustia*, *Guarea*, and the *Myrsine guianensis* group must have been originated in this way.

The broad-leaved semi-deciduous Yucatan flora

It strikes one that there are numerous species common to the semi-deciduous forests of lowlands and hill-countries in western Cuba and Yucatan. Some of them occur solely in the Guanahacabibes peninsula and Yucatan. As examples the genera *Hirtella*, *Poiretia*, *Eriosema*, *Belotia*, *Luehea*, *Schwenckia*, *Chimarrhis*, *Calycophyllum*, *Elaeagia*, *Deherainia*, *Ateleia*, *Forchhammeria*, and *Neomacfadya* are worth mentioning. Although the presence of these elements suggests a direct relationship between the Miocene flora of Mexico and Cuba, there are neither geological nor biogeographical evidences supporting the existence of land connections in that period.

Elements from the temperate North America

The next most important region influencing the flora of Cuba, particularly western Cuba, is Florida and the southeastern United States. Several elements, such as *Pinus*, *Quercus*, *Fraxinus*, and many species of Ericaceae, Gramineae, and Cyperaceae, etc., of the temperate zone immigrated from the north and became predominant in some parts of western Cuba. ALAIN (1958 p. 19) presumes the existence of a former land connection between western Cuba and Florida, although according to geologists this possibility has been excluded since the Eocene Epoch. In my view, the flora from the temperate zone in western Cuba developed during the glacial periods in the Pleistocene, that took place simultaneously in the whole continent as pointed out by VAN DER HAMMEN (1961) and VEILLEUMIER (1971). Consequently, instead of assuming a land strip it is more reasonable to trace back the Florida-Western Cuba relationship to the Madro-Tethyan flora. As already seen, this sclerophyllous evergreen flora originally contained several genera from the families of the temperate zone. In addition, there was sufficient amount of time during the Miocene available for a fairly uniform vegetation to develop, first on the coast of the Tethys Sea and later, following the emergence of Central America, along the Gulf of Mexico. This flora was exposed to the cooler climatic periods occurring many times in the Pliocene and the Pleistocene. In particular, the coastal region of the Gulf of Mexico was affected, since the cold Labrador Current often reached this zone during the Pleistocene. The new environmental conditions, e.g., modified soil types, and the adaptive responses of plant populations generated by this cooling process were probably similar on both sides of the gulf. The standardization of the flora may have been furthered, too, by the strong northerly and westerly winds during the glacial periods and by the more intensive bird migration. In Cuba, the expansion of this flora was also promoted by the newly emerged lands and white sands that offered extensive bare areas for plants. Thus, the recent flora elements common to western Cuba and Florida are remnants of a Pleistocene

flora spread along the entire coast of the Gulf of Mexico. The continuity of this flora was broken in the postglacial period, when the Central American subtropical flora retreated to the western coast of the gulf, forcing back the remnants of the Madro-Tethyan flora to a north-western and northeastern direction.

Remarks on the driving forces of migration

In connection with the above discussion, it has to be emphasized that very little is known about the water and aerial dispersal of seeds. Seed dispersal is likely to be more important than generally thought, and the explanation of floristic relationships does not necessarily require a search for disappeared land strips. Only confirmed geological results may serve as a basis for hypotheses on the distribution, development and migration of the flora. Moreover, even undoubtful land connections may prove to be of no help in promoting dispersal. For instance, *Befaria cubensis*, *Microcycas calocoma*, and *Rhus copallina*, did not reach Isla de Pinos despite the direct terrestrial contact to Cuba in the Pleistocene. Although *Fraxinus caroliniana* ssp. *cubensis* produces easily flying seeds, it could not become established on moorlands outside the Zapata peninsula. Many genera (e.g., *Victorinia*) and species (e.g., *Omphalea commutata*) are still restricted to an area along the former contact line between Cuba and Hispaniola (Fig. 21) although the ecological conditions would allow a larger geographic range on both islands. Many elements of the Bahamas, that managed to get across the wide and deep Bahama Trench and became established on the reefs around northern Camagüey, Tunas and Holguín, were unable to penetrate into Cuba through the narrow and shallow bays and even via the former land strips. Consequently, a terrestrial contact may not necessarily be enough by itself and cannot be a single explanation for migration. The migratory activity of taxa does not always coincide in time with the possibilities. In other cases unfavourable ecological and cenological conditions, such as the saturation of the flora, may prevent otherwise feasible migration.

Flora migration in the interior of Cuba

The chorological groups reflecting the origin of the Cuban flora, the approximate time of their manifestation and the potential migratory routes have already been discussed. These are, however, only the initial steps toward

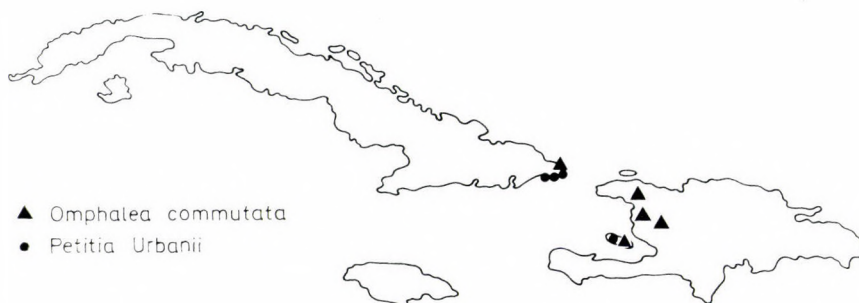


Fig. 21. Geographical distribution of *Omphalea commutata* Muell. Arg. and *Petitia Urbanii* Ekm. (after ALAIN 1972 and BORHIDI 1973)

a complete flora development. The actual floristic composition is a result of further differentiation, adaptation and internal migration. Two fundamental principles should be considered in this regard.

Two principles of internal floristic changes in Cuba

A. Oriente is the earliest cradle of the Cuban flora, a starting point for the most important migrations.

B. The floras of mountains are older than those in the lowlands. The flora and vegetation of plains are originated from the mountainous regions.

Three stages of internal migration

According to time, starting point and direction, three stages of internal migration can be distinguished.

a. *Migrations in the early Tertiary* (Eocene to Miocene Epochs). The centre of these movements, that were directed from the east to the west, was the mountains of Oriente. The low differentiation and specialization level of the flora is typical of this stage. Several migratory waves may be recognized, these are associated with different periods. The radius of action was determined in each case by geological events, transgression and the emergence of lands, that actually happened. The serpentine and karst flora of western Cuba and then the montane flora of the Escambray Mts were originated in this way. Later, the submontane tropical flora, which subsequently colonized the rising plains, took refuge in the valleys.

b. *Migrations at the end of the Tertiary and in the Quaternary* (Pliocene and Pleistocene Epochs). Starting from both tips of the island the migrations were directed toward the interior. As a result, a new flora in the central plains of Cuba became established at the end of the Pliocene. The migrations of alternate direction in the Pleistocene are also mentioned here. During cooler periods these migratory waves swept over Cuba from the west to the east, but in the warm periods the migration was of the opposite direction. This is the time of ecological and cenological adaptation and specialization. The vegetation zones and, among others, the formation of the endemic montane flora of Turquino may also be dated back to this stage. Furthermore, the homogenization of the flora and vegetation, that were originated from different mountains, may have been caused by the repeated "shake up" of the flora of plains.

c. *Migrations driven by anthropogenic forces during the historical ages*. The starting point for these migrations is usually western Cuba, so the direction is toward the east. The reason for this is that western Cuba was the sanctuary of many savanna and prairie elements in the Pleistocene, that found refuge particularly in the herb layer of coniferous forests. The migration of these plants was made possible by tropical deforestation, which is mainly responsible for secondary savannas and the recent landscape of Cuba. In addition to the migration of native plants, many species were introduced most recently by man. These elements, too, migrate usually from the west to the east.

Evolutionary centres and the migration of ecological groups

The evolutionary centres and the migratory characteristics of genera, families and sections may be explored by comprehensive morphological and microevolutionary studies at the given taxonomic level. However, only a few relevant works have been published, for instance, WEBSTER (1958) on *Phyllan-*

thus, JUDD (1982) on *Lyonia*, DAHLGREN and GLASSMAN (1963) on *Copernicia*, and HOWARD (1949) on *Coccoloba*. Of course, besides the analysis of taxonomic categories, the evolutionary centres and migratory pathways of life forms or vegetation types may also be revealed by thorough studies. For example, the mangrove communities reach the highest diversity in the Indonesian archipelago, which may be considered the evolutionary centre of the mangrove vegetation types (ANDREÁNSZKY 1954). The localization of the richest and the poorest stands of each vegetation type or plant community in Cuba may be readily determined based on phytosociological relevés and vegetation maps. In the present study relevés taken by BORHIDI, MUÑIZ, DEL RISCO, CAPOTE and SAMEK are used together with published (LEÓN 1936, 1939, MARIE-VICTORIN 1942, 1944, 1956, WEBSTER 1956–58, BORHIDI and MUÑIZ 1977, KLOTZ 1979, and BORHIDI 1972, 1981) and unpublished (BORHIDI) distribution maps.

Evolutionary centres and migratory routes of the serpentine vegetation

The distribution of some typical elements of the serpentine vegetation is shown in Figs 8, 9, 11, 22. The evolutionary centres and principal migratory routes are illustrated in Fig. 23. The section *Orbicularia* of the genus *Phyllanthus* is a typical serpentinophilous group that includes many species occurring on the old ferritic soils of northeastern Oriente. Of this section *Ph. orbicularis* is the only species that is found on all the serpentine outcrops of the island (Fig. 8). Like *Neobracea valenzuelana* (Fig. 9), this species must have reached its suitable habitats along the route indicated by arrows in the figure. LEÓN (1946) and WEBSTER (1958) assumed that this migration took place along a continuous serpentine "axis" ("eje serpentinoso"). Such a serpentine strip could not have existed later than the Oligocene, due to the long transgression of central Cuba. It is doubtful, however, whether such a large continuous serpentine area could have been denuded by that time, although the distributions of vicarious genera (Fig. 10) and species (Figs 11–12) of the Nipe Mts and Cajalbana suggest that a direct relationship may have existed. Yet, according to our recent geological knowledge, it is more likely that the serpentine blocks were more or less separated all the time. The serpentinophilous species were presumably distributed either by wind (*Neobracea*) or by animals (*Phyllanthus*). They may have had wider distribution earlier, but due to the depletion of habitats (KRUCKEBERG 1954) they survived only on serpentines and became subjects of intensive speciation. It seems that there were several stages of the east-west migration of the serpentine flora. Taxa with strongly disjunct geographical range, e.g., *Anemia*, *Moacroton*, *Lescaillea*, and *Harnackia*, etc., were distributed during the earlier stages. Later, some serpentinophilous species, e.g., *Jacaranda cowellii*, *Coccoloba geniculata*, *Zanthoxylum nannophyllum* (Fig. 22), and *Platygyne parviflora* (Fig. 14), migrated replacing the above mentioned taxa in the plains of eastern Cuba. In these stages the species with continuous distribution, such as *Neobracea valenzuelana* and *Phyllanthus orbicularis*, became also widespread. On the basis of all these, three evolutionary centres of the serpentine flora may be distinguished in Cuba (Fig. 23). The first and the oldest is Moa from which the serpentine flora of the entire island, and the flora of other ferritic soil areas in northeastern Oriente, the Baracoa-Jauco zone and the Cristal and Nipe Mts were originated. The second is Nipe, a migratory centre of a more xerophilous serpentine flora which was capable of being established in the arid lowlands and of migrating to the west. The third centre is Cajalbana in western Cuba. A part of its flora retreated

to the serpentine blocks risen later in central Cuba, especially to Habana and Matanzas. Many latosol elements of this flora became adapted to the acidic slaty habitats of Pinar del Rio and Isla de Pinos. In Fig. 23 the dotted line directed to the east indicates the secondary migration of serpentine elements from areas which subsequently became secondary savannas under the influence of man.

Evolutionary centres and migratory routes of the flora of limestone cliffs

The flora of limestone karsts has two primary evolutionary centres (Fig. 24). One of them is in the conical karsts of Sierra de los Organos in western Cuba, which is considered to be the oldest geological formation on the surface of that area. The majority of species found here remained endemics, although a portion of the flora spread to the younger Tertiary karsts in the Habana and Matanzas Heights, e.g., *Bombacopsis cubensis*, *Thrinax morrisii* (Fig. 14) and *Phania matricarioides* (Fig. 25). Certain elements managed to get dispersed as far as to the high-altitude mogotes and to the southern coast at the Escambray Mts. During the transgression, Cuba itself was an old coastal zone serving as a refuge of the vegetation of coastal karsts. This vegetation started to spread over the recent coastal zone at the end of the Tertiary. The other primary evolutionary centre is the mountains of Oriente. Here, the development of the karstic flora is not as clear-cut as in western Cuba, since the karsts of Oriente are younger than the serpentine formations and, also, a part of the limestone flora is originated from primary soils derived from serpentine (e.g., Nipe Mts, Monte Libano). Of the many isolated

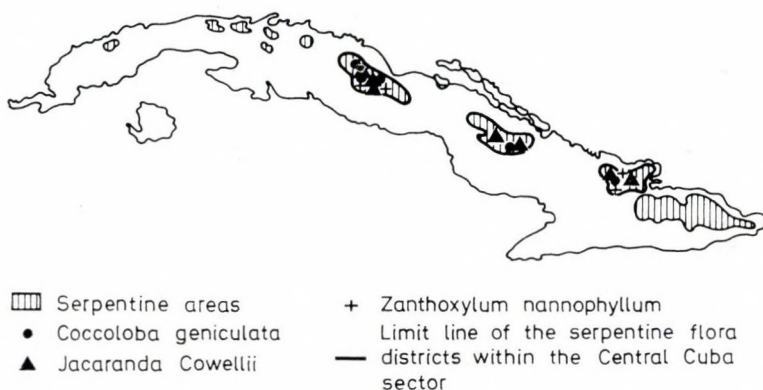


Fig. 22. Geographical distribution of *Coccoloba geniculata* Lindau, *Jacaranda cowellii* Britt. et Wils. and *Zanthoxylum nannophyllum* (Urb.) Alain (after BORHIDI 1973)



Fig. 23. Evolution centres and migratory routes of the serpentine flora in Cuba (after BORHIDI 1973)

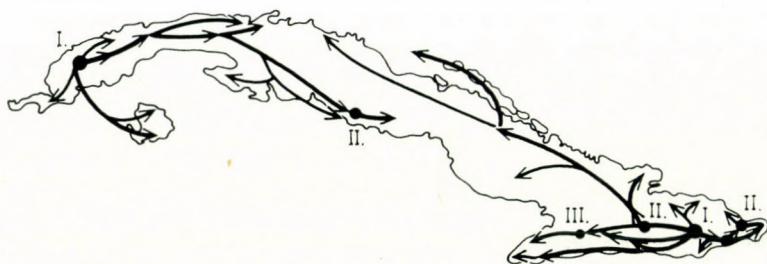


Fig. 24. Evolution centres and presumable migratory routes of the flora of limestone karsts in Cuba (after BORHIDI 1973)

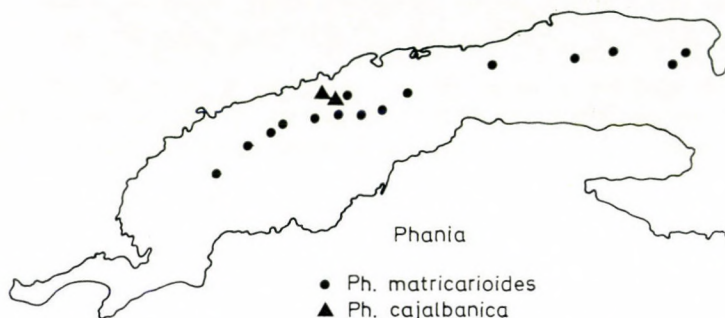


Fig. 25. Geographical distribution of the genus *Phania* (after BORHIDI 1973)

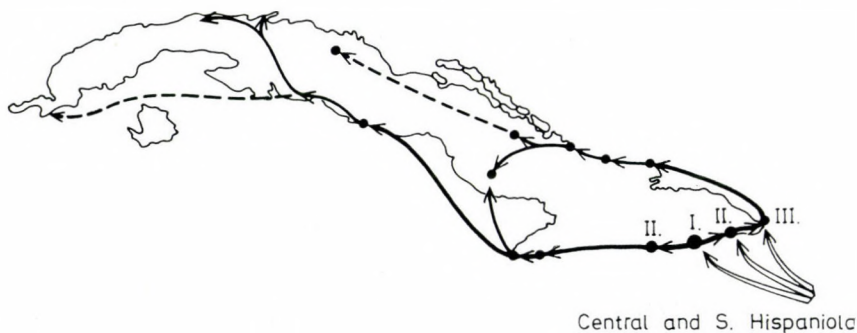


Fig. 26. Possible centres of evolution and migratory routes of the subdesertic, xero-megathermophilous elements of Cuba (after BORHIDI 1973)

karstic regions, the group of Monte Libano and Monte Verde appears the most peculiar evolutionary centre, so much the more because this area was crossed by many migratory paths of varying nature and direction. From this old coastal karstic zone spread the karst flora over the country, first along the coastline. In the westerly direction two secondary evolutionary centres developed: the karsts southwest of the Nipe Mts, and at the northern border of Sierra Maestra. In both areas the montane elements descending from the mountains were mixed with the karstic flora spread along the coast. The influence of this flora was extended to the east as far as the Yumuri karsts and Yunque de Baracoa, with a secondary evolutionary centre developed in the latter place. The rise of the terraces in southern Baracoa made possible

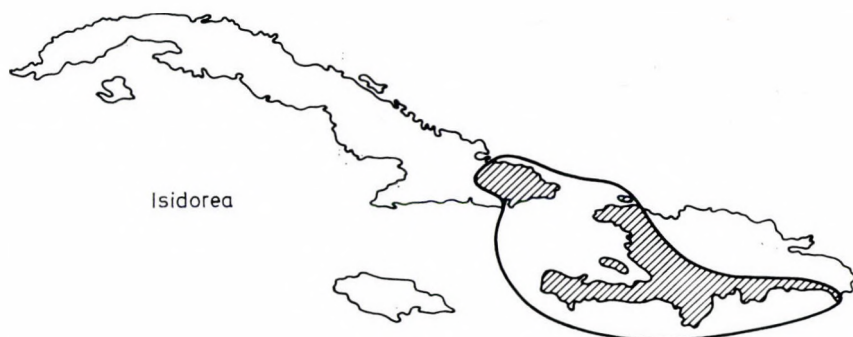


Fig. 27. Geographical distribution of the genus *Isidorea* A. Rich. (after BORHIDI 1973 modified)

for the flora of Monte Libano to spread over the semi-desert coastal zone. As a result of the migration, xero-megatherm species evolved. Some elements of the Oriente karstic flora migrated along the southern coast at the Sierra Maestra. The others spread to the west from the Nipe Mts via the former limestone block mountains along the northwestern coast of central Cuba, forming the flora of the Cubitas and Najasa Mts and, too, the flora of coastal shelves. The influence of this migration may be seen even in the mogotes of Caguagas (cf. the distribution of *Hemithrinax*, Fig. 13). This bidirectional distribution exhibited by karstic elements is an important characteristic of the recent flora, too, so it was considered in the delimitation of the flora regions of central Cuba.

Evolutionary centres and migratory routes of the xero-megatherm elements

Some semi-desert xero-megatherm elements are originated from central and southern Hispaniola (Fig. 26), to which the southeastern coast of Cuba was formerly connected. This relationship is proved by the distribution of several genera and species, e.g., *Omphalea comutata*, *Petitia urbani* (Fig. 21), *Victorini*, and *Isidorea* (Fig. 27), now restricted to the coastline. These elements, thanks to the gradual rising of the southern Baracoa coast, were intermixed with the endemic flora of karstic slopes. As a result, several centres of speciation developed, the richest of them being the Guantanamo Basin. The flora of Macambo-Imias and Maisi, and the Daiquiri-Siboney-Santiago area in the west are almost as rich as that of the Guantanamo Basin, so they can be considered as secondary and tertiary evolutionary centres, respectively. The number of xero-megatherm elements on the southern coast at Sierra Maestra, on the coasts of northwestern Oriente and northern Camagüey, in the southern foothills of Escambray Mts, on the northern rocky coast between Habana and Matanzas and on the southern limestone shores of the Guanahacabibes peninsula decreases in that order.

Evolutionary centres and migratory routes of the montane rainforest elements

The evolutionary centres and migratory routes of the flora in the montane rainforests are shown in Fig. 28. The distribution of *Hedyosmum* serves as an example (Fig. 7). Again, the main centre is the lower and old mountains in the Sagua-Baracoa Massif, especially the Moa-Toa area. According to my hypothesis, this region received its montane elements via Hispaniola, and served as a new centre for their further migration to Cristal, Nipe, Purial,

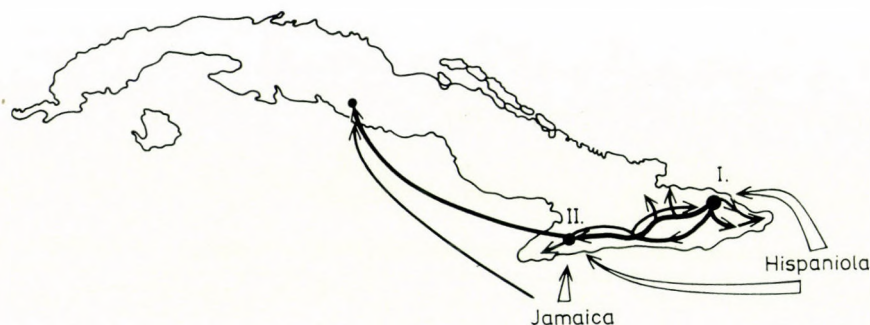


Fig. 28. Centres of evolution and possible migratory routes of the montane rain forest elements in Cuba (after BORHIDI 1973)

Imias mountains, and, in particular, to Sierra Maestra. The first stage of this process took place presumably in the mountain-range connecting Oriente and Escambray. The more permanent changes, however, are results of migrations in the cooler periods during the Quaternary between Sierra del Cristal and Gran Piedra through the Central Valley area which subsided later. At that time the lower border of montane rainforests may have run at a 2–300 m altitude. This montane flora was mixed with Jamaican and southern Hispaniolan montane elements and, during the cool stages in the Pleistocene, may have repeatedly retreated to the subalpine-alpine zone in the Turquino group. In this place a secondary centre of speciation developed. It is likely that even at that time some montane elements of Sierra Maestra got across to the Escambray Mts, which received elements from the montane flora of Jamaica, too.

Distribution centres and migratory routes of the semi-deciduous elements

The origin and migration of the elements of semi-deciduous forests and seasonal rainforests in the lowlands pose different problems. ALAIN (1958) traces the origin of some elements back to Mexico, assuming a west-east migration of these forests. It is my conviction that this flora survived in the refuge of valleys at the time of transgression, and subsequently spread over to every direction. Especially the mountains of Oriente, northern Camagüey, Pinar de Río and the Escambray Mts should be mentioned as potential refuges. The high similarity between the characteristic composition of lowland and submontane forest formations also refers to this fact.

For cited references see the second part.

SOME PROPERTIES OF RAINFALL AND THROUGHFALL WATER IN UNDISTURBED JUNIPER AND POPLAR FORESTS IN BUGAC

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Field studies were conducted to examine the amount and some chemical properties of the precipitation in an open plot and in adjacent poplar and juniper forest stands in one vegetation period on the Great Hungarian Plain.

The data are discussed, with particular emphasis on the selection of sampling sites and periods between subsequent samplings. A considerable difference between the canopy interception properties of juniper and poplar was found. Excess acidity beyond CO_2 saturation was found to be negligible. Comparison of Cl^- -amounts collected on open plot and under canopy indicate intensive dry fallout. From these observations, a relation between canopy interception and forest floor vegetation patterns is expected.

Introduction

Extensive ecological research has been undertaken by the Institute of Plant Taxonomy and Ecology at Eötvös University in the Bugac biosphere reservation of the Kiskunság National Park. Part of the research is an investigation of the pattern of communities and vegetation (SIMON et al. ined.). One major point in the development of the pattern is the water and nutrient budget of the soil, as is typical for arid and semi-arid areas.

Generally, water supplies to the soil are atmospheric precipitation, surface and sub-surface interflow and groundwater. Water drainage of the soil is controlled by evapotranspiration, surface and sub-surface interflow and deep seepage.

As a consequence of its soil characteristics, surface topography and climate, the observed area exhibits a deliberately simpler water-balance behaviour. The groundwater is much too deep to contribute to the water balance. (The groundwater level is generally 2-6 m below the surface.) The sand is rather porous, so that virtually all incident precipitation percolates through the soil and no surface run of water was ever observed. Only a minor, almost negligible downhill movement can be expected from sub-surface interflow. As a result, we can consider atmospheric precipitation to be the only water input of the research area, and the only output to be evapotranspiration.

In forested areas, the amount and chemical composition of the rainwater reaching the soil-surface is quite different from that in open plots. The main aim of our investigations is to observe the amount and the nutrient content of the rainwater during the vegetation period and its variabilities under the forest canopy.

This paper is a report covering the measurements on the amount and some chemical aspects of atmospheric precipitation and canopy throughfall in juniper and poplar forests, made between March and October, 1983.

Our observations served as preliminary testings of the sampling sites and methods, the choices of periods between subsequent samplings and, finally, the electrochemical methodology.

The first part of the paper gives details of rainwater investigations and data processing, while the second part contains the conclusions of precipitation studies.

Site of observation

Bugac is one of the MAB plots in Hungary. The experimental area is located between the rivers Danube and Tisza, about 30 km from Kecskemét in a south-west direction (Fig. 1).

The average annual precipitation in the research area ranges between 530–550 mm. The 1983 year was extremely dry, the precipitation amount was only 368.7 mm (Fig. 2). The region has a mean annual air temperature of 10–11 °C and there is a wide range of both daily and annual temperatures.

Soils are mostly slightly humous sandy soil with a thin organic layer (5.0–10.0 cm) on the surface, with a maximum humous content of 1.0%.

Semi-closed sandy grassland (*Festucetum vaginatae danubiale*), juniper (*Juniperus communis*) and poplar (*Junipero-Populetum albae ligustretosum*) are the most widely occurring communities in the area.

Sampling and analyses

We used 23 standard Hellmann-type gauges of 200 cm² surface as rain collectors, with their openings about 1 m above ground. Four of them were located on an open plot, eight under the canopy of some *Juniperus communis* (hereafter referred to as juniper), and eleven under the canopy of a forest consisting mainly of *Populus alba*, *Populus canescens* and *Ligustrum vulgare* (hereafter referred to as poplar).

Sampling was done weekly or after longer periods. We often succeeded in collecting samples after storms. On several occasions, we collected the precipitation of one storm, immediately after it had ceased. In Fig. 2, the individual sampling times are marked on a climate diagram from the nearby Kiskunfélegyháza Meteorology Station.

Precipitation samples were stored in polyethylene flasks. The collected amount of water (in mm), the Cl⁻ content (in ppm) and the pH-value of each of the numbered gauges were measured and recorded. There were several reasons for choosing these two chemical components during our preliminary observations.

The first point is that field analysis can only be made by simple electrochemical methods using ion-selective electrodes. Even so, special care must be taken, because the ionic strength and the concentration of disturbing (interfering) ions are difficult to control. Using the present analyses as a basis we would like to measure NO₃⁻, NH₄⁺, and alkali-ions, as well. Acidity measurements are of prime environmental interest, and the change in H⁺ concentration is the greatest among the chemical components interfering with forest canopy. On the other hand, Cl⁻ represents non-interfering (or only to a negligible extent) chemical components.

A RADELKIS OP-Cl-0711P type AgCl electrode was used for the Cl⁻ measurements, and an OP-0718P type glass electrode for the pH-measurements. An OP-0821 type double junction Ag/AgCl reference electrode was used for both measurements, the outer chamber filled with 1 M (NH₄)₂SO₄ solution. Electrode potentials were pre-amplified by a self-made amplifier with an input impedance of 10¹² Ω, and measured by a MINIMULTI MM 2002 type digital voltmeter, with an accuracy of ±1 mV. The whole device operated by battery, completely independent of the mains. Voltages were displayed and recorded and concentration and pH values were always calculated. The 99% confidence intervals for Cl⁻-concentration and pH were max. ±2.8 rel.% and ±0.05 pH-unit, respectively. A detailed description of the measurements and calibrations is reported elsewhere (KESZEI and SZABÓ 1984).

Summary statistics

Here we discuss the means and variances (s_x^2) of different variates and samples, and the correlation matrices of the variates. Some important estimates are summarized in Tables 1 and 2.

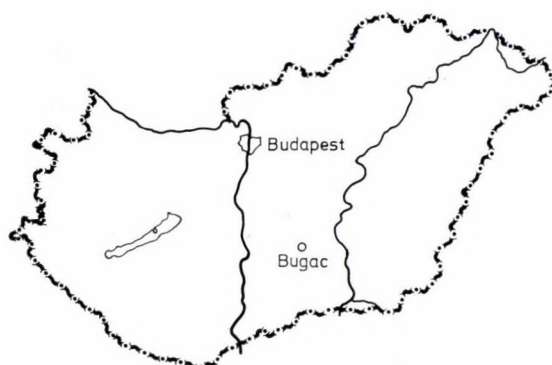


Fig. 1. Situation of Bugac

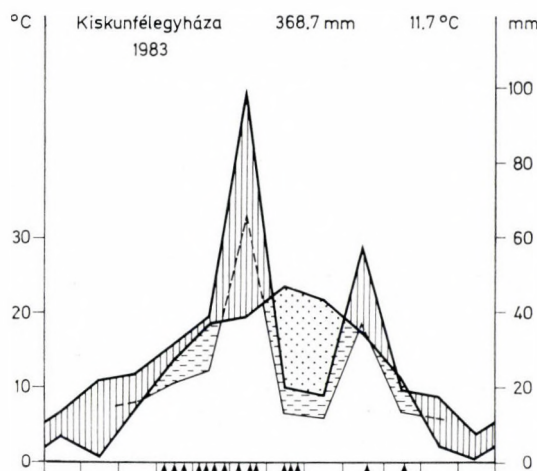


Fig. 2. Climatogram of the Kiskunfélegyháza Meteorology Station (17 km from Bugac). Arrows indicate dates of sampling

The uniform standard deviations of the means ($s_{\bar{x}}$) of samples collected in various plots are remarkable. According to these, all samples may belong to the same type of distribution having the same variance and only different means.

If we consider the throughfall in relation to the open plot amount of precipitation, the means for juniper and poplar are different, although their respective 95% confidence intervals overlap. The smaller standard deviation of the poplar might indicate the greater homogeneity of the poplar canopy density.

Both the concentration and the amount of Cl^- ions increases during throughfall, although the former is expected due to the evaporation from the canopy. The H^+ content of the throughfall water decreases dramatically compared to the open plot precipitation. Open plot pH-values are close to those of CO_2 -saturated water, and only a slightly higher acidity can be detected. Both forests neutralize this acidity, partly through CO_2 -transpiration and partly through the ion-exchange and buffer capacity of the leaves.

A remarkable feature of the correlation matrix, shown in Table 2, is that, besides the amount of throughfall water, its chloride content also exhibits a great, positive correlation with the amount of open plot precipitation ($r = 0.98$ and $r = 0.93$ for poplar, respectively).

Table 1

Summary statistics of some of the observed variables. Values in brackets are uninformative, though not ignored. C denotes concentration in the collected rainwater and m its quantity per m²

| Means | | | Variables | Standard deviations | | | Number of samples |
|---------|---------|--------|---|---------------------|---------|--------|-------------------|
| Poplar | Juniper | Open | | Open | Juniper | Poplar | |
| (18.8) | (18.8) | 18.8 | rainfall, mm | 16.8 | (16.8) | (16.8) | 14 |
| 12.8 | 12.0 | (18.8) | throughfall, mm | (16.8) | 16.8 | 16.4 | 14 |
| 58.0 | 48.6 | (100) | throughfall, % | (89.3) | 23.2 | 15.3 | 14 |
| 6.63 | 10.1 | 2.32 | C _{Cl⁻} ppm, | 0.69 | 4.8 | 2.4 | 9 |
| 78.1 | 100.1 | 43.7 | m _{Cl⁻} mg/m ² * | 51.3 | 128.0 | 89.0 | 9 |
| 6.41 | 5.89 | 5.46 | pH | 0.84 | 0.22 | 0.30 | 6 |
| 0.00068 | 0.0029 | 0.0124 | C _{H⁺} ppm | 0.0180 | 0.0023 | 0.0008 | 6 |
| 0.0082 | 0.0240 | 0.1380 | m _{H⁺} mg/m ² * | 0.2170 | 0.0250 | 0.0150 | 6 |

* 1 kg/ha = 100 mg/m²

Table 2

Correlation matrix of some of the variables. Correlation coefficient values are percentages, mm stands for rainfall in mm, m_{Cl⁻} for the Cl⁻-amount of rainwater in mg/m²

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|-----|-----|-----|-----|-----|-----|-----|-----|
| 1. Open, mm | | 92 | 98 | 84 | 93 | -64 | -20 | -91 |
| 2. Juniper, mm | 92 | | 97 | 78 | 85 | -60 | -10 | -88 |
| 3. Poplar, mm | 98 | 97 | | 81 | 89 | -67 | -20 | -90 |
| 4. Juniper, m _{Cl⁻} | 84 | 78 | 81 | | 98 | -22 | 35 | -69 |
| 5. Poplar, m _{Cl⁻} | 93 | 85 | 89 | 98 | | -36 | 14 | -80 |
| 6. Open, m _{Cl⁻} | -64 | -60 | -67 | -22 | -36 | | 65 | 50 |
| 7. Open, pH | -20 | -10 | -20 | 35 | 14 | 65 | | 74 |
| 8. Poplar, pH | -91 | -88 | -90 | -69 | -80 | 50 | 74 | |

The open plot pH values exhibit low correlation values, but their independence is expected due to the above-mentioned CO₂-saturation. The high negative values with regard to the pH of the water in poplar reflect the fact that, the greater the amount of precipitation, the less the efficiency of the neutralization by leaves.

To test the choice of the sampling sites, summary statistics of the numbered gauges as variables were calculated, while sampling times served as replicates. Ignoring the reports of these numerous tables of 23 × 14 data, let us only make some remarks. It turned out that one of the four open plot gauges always recorded a significantly smaller amount than the others, so we suspect its unreliability.

The high correlation between the amount of Cl^- and the amount of precipitation ($r > 0.95$) is independent from the site of the gauges. Correlations in juniper are smaller than in poplar, except for a single gauge which sometimes even exhibited negative values with other gauges. This "outlying" gauge was located too far from the others in poplar, mainly under *Ligustrum vulgare*, on the boundary of poplar area.

Between gauges in poplar and in juniper, there is a small correlation ($r < 0.6$) regarding chemical properties. Different behaviour in influencing chemical properties of throughfall water is therefore expected between juniper and poplar areas.

To test the time intervals between subsequent samplings, we made similar calculations. This time the dates of samplings were regarded as variables and the 23 gauges as replicates. With respect to the collected amount and the Cl^- concentration of water, samplings can be arranged into two groups which overlap slightly. The pH data does not contradict this arrangement. One group contains 9 of the 14 samplings, collected within a fortnight following the previous sampling. The other group contains samples collected in three to five week intervals and greater amounts of water, except for one sample (17th July), which contained only 7.5 mm water from a single storm with a rather strong wind, lasting merely 1.5 hours. However, the period between the samplings might have had an influence on the chemical properties of the rainwater.

Relations among observed and derived variables

1. Chemical properties

Let us consider here some of the relationships already suspected with regard to summary statistics. Firstly, we will consider the close correlation of all the Cl^- amounts collected on the different sites. The calculated significance probabilities of the linear relationship between the amount of Cl^- in precipitation water in open plots and the amount of collected Cl^- under canopy were usually about 0.0001% for individual gauges, and never less than 0.01%. Furthermore, the straight line through the origin (i.e. the homogeneous linear relation) fits better to the data than a general straight line (see e.g. GREEN and MARGERISON 1978).

So the gross amount of Cl^- in throughfall water is *directly proportional* to the gross amount of Cl^- contained in open plot precipitation water. The 95% confidence interval for the weighed averages of the proportionality factors of respective gauges is 2.4 ± 0.2 in the case of juniper and 1.75 ± 0.14 in the case of poplar. It should be noted, however, that correlation coefficients between the open plot and throughfall Cl^- concentrations do not exceed 0.8.

In Table 2, we see a negative correlation between Cl^- concentration and the amount of precipitation on the open plot, but there is no evidence of linear relationship. Figure 5 shows the plotted data points for 6 samplings with small precipitation amounts. This scatter diagram does not suggest any functional relationship. However, on the basis of literary data in this field (MADGWICK and OVINGTON 1959), a steadily decreasing function is expected. The most general form of this relation is the

$$y = a + b \cdot e^{cR} \quad (1)$$

type exponential, where y denotes the concentration, a and b are constants of concentration dimension, R = gross precipitation, and c is a dimensionless constant. However, the fit of type (1) function is rather poor, as the sum of squared residuals exceeds the total sum of squares around the mean. Nevertheless, the trend of the fitted line, as turns out from its parameters ($a = 1.4$ ppm, $b = 3.7$ ppm, $c = -0.125$), indicate relatively great atmospheric Cl^- -sources, anthropogenic or natural (WOLAVER and LIETH 1972).

2. Rainfall interception

An interesting relationship is expected between the amount of open plot precipitation and the gross throughfall. The calculated significance probabilities of a linear relationship are less than 0.0001% for all gauges! Many papers (SKAU 1964; ZINKE 1967; THOMPSON 1972; ETTEHAD et al. 1973; and GASH 1979) usually interpret this linear relationship by counting the minimum amount of precipitation necessary to start throughfall. This amount (usually 1–2 mm) is called “canopy saturation”. If we calculate this type of canopy saturation using our highly correlated data as a basis, we generally find 2 ± 2.5 for the 95% confidence intervals, including zero and even negative values. We note that other reported confidence values — if any! — agree with our results (SKAU 1964). There is also another quibble with the linear relationship. If the relation between gross precipitation (R) and throughfall (T) is

$$T = a + bR, \quad (2)$$

then the interception ($I = R - T$) is given by two intersecting lines:

$$I = R, \text{ if } R < \text{canopy saturation} \quad (3)$$

$$I = -a + (1 - b)R, \text{ if } R \geq \text{canopy saturation} \quad (4)$$

However, the change-over from (3) to (4) cannot be as acute as the above, especially if we take into account the fact that interception, free throughfall, dripping and evaporation from canopy occur simultaneously from the start of the rain on.

A more adequate approach to this problem was put forward by CZARNOWSKI and OLSZEWSKI (1968), who described canopy interception using

$$I = I_{\max}(1 - e^{-cR}), \quad (5)$$

where I_{\max} is the maximum possible gross interception in mm, and c a dimensionless measure of the interception ability. The solid line in Fig. 3 shows this function fitted to the interception data points for poplar. The goodness of the fit is highlighted in the R^2 values,* which are 0.81 for the straight line and 0.96 for the exponential curve.

Figure 4 shows the derived relation for throughfall:

$$T = R - I_{\max}(1 - e^{-cR}). \quad (6)$$

The R^2 value for the broken line is 0.985, while, for the fitted solid curve [according to equation (6)], it is 0.994. We note that the fit of the exponential line to our data points observed in poplar is better than that of the reported data (from 100 gauges) in CZARNOWSKI and OLSZEWSKI's original paper. The fit of a type (6) curve to the juniper data points is not as good as in the case of poplar ($R^2 = 0.36$ for the interception and $R^2 = 0.92$ for throughfall). However, they both exceed the R^2 values for the respective linear relationship. The parameters of the fitted curve for poplar are $I_{\max} = 9$ mm and $c = 0.07$, which is in surprising accordance with CZARNOWSKI and OLSZEWSKI's 10.2 mm and 0.06.

* $R^2 = 1 - \frac{\text{SSR}}{\text{SST}}$, where SST is the total sum of the squares around the mean and SSR is the sum of the squared residuals around the fitted curve. R^2 is sometimes called the determination coefficient. For a linear relationship, R^2 equals the square of the correlation coefficient.

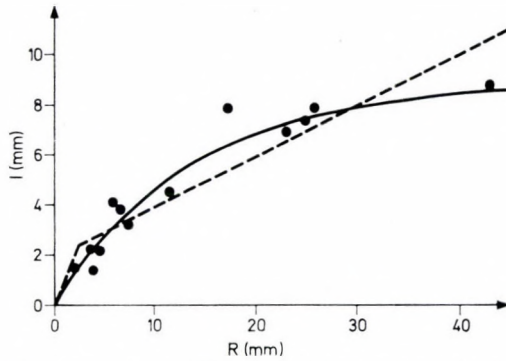


Fig. 3. The intercepted water on canopy (I) vs. open plot precipitation (R). Solid circles denote observed data in poplar stand. The solid line is the fitted type (5) curve. The broken line is a fitted type (3) and (4) curve

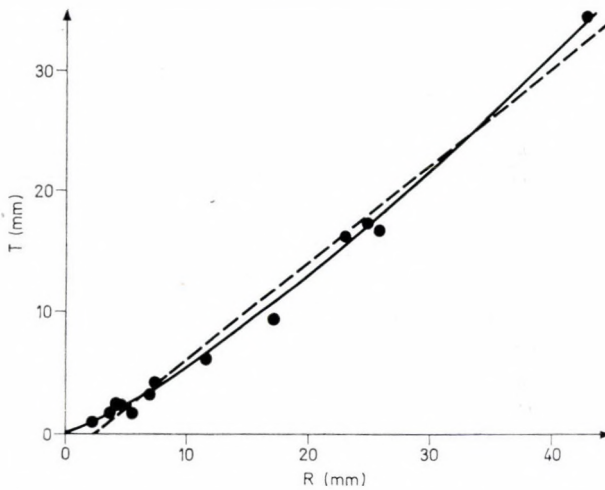


Fig. 4. Canopy throughfall (T) vs. open plot precipitation (R). Solid circles denote observed data in poplar stand. The solid line is the fitted type (6) curve. The broken line is the regression line

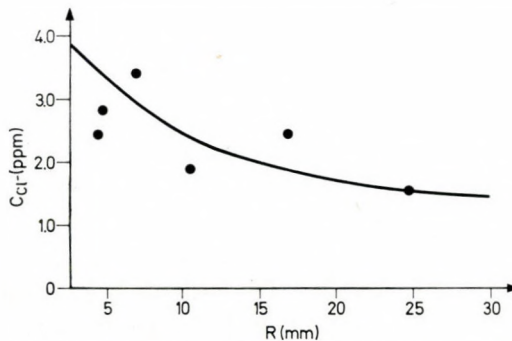


Fig. 5. Cl^- -concentration of open plot precipitation vs. its height in mm. Solid circles denote observed data. The equation of the solid line is $c = 1.4 + 3.7 [\exp (-0.125 R)]$ ppm

With regard to Table 1, there arises the question if there is a significant difference between the throughfall amounts in juniper and poplar. As the standard deviations are the same, we made a rank-sum test. Using the Mann-Whitney statistics, the difference is not significant ($P_\alpha = 34\%$).

Results and discussion

The sum of the collected precipitation during the observation period is as follows:

| | |
|----------------|---------|
| open plot: | 263 mm, |
| under juniper: | 168 mm, |
| under poplar: | 179 mm. |

Total gross interceptions over the same period are:

| | |
|-------------|--------|
| in juniper: | 95 mm, |
| in poplar: | 84 mm. |

From type (5) and (6) functions, i.e. the fitted curve, we can conclude that there is a basic difference in interception between juniper and poplar. First, the maximum possible interception capacity of 6.5 mm in juniper is less than the respective 9 mm in poplar. On the other hand, the constant c , which characterizes the slope of the curve, is 0.16 in juniper, i.e. more than twice the value of poplar (0.07). The consequence of this is that, in the case of smaller storms (less than about 15 mm precipitation), juniper intercepts more water than poplar does, while in the case of greater storms—because of its greater maximum capacity—poplar is less transparent for the gross throughfall than juniper is. This is probably a consequence of the different shapes and surfaces of the respective leaves.

However, this situation can be changed by some meteorological factors. Wind velocity seems to play a paramount role in this change, mainly due to its direct “shaking off” of water from the canopy. Wind direction also has a great influence on boundary positioned gauges. As juniper plots are relatively small (and rather stinging!), there are more boundary positioned gauges in juniper than in poplar. This makes the standard errors greater, and the fit of type (1) and (6) curves less reliable, for juniper. We will put some more gauges within juniper plots to avoid this wind sensibility.

The above difference in the interception of the two forest-types has a direct influence on the forest floor vegetation. Storms yielding less than 6–8 mm precipitation fall through the juniper canopy in only a very small amount, while the net precipitation under a poplar canopy is considerably more. This could have an important effect on the vegetation pattern.

As the observation period was relatively dry, we observed greater total throughfall values in poplar rather than in juniper. In periods of greater precipitation, this relation often reversed.

The results of the Cl^- measurements are surprising. The dependence of the concentration of Cl^- on the amount of precipitation in an open plot indicates abundant Cl^- sources in the atmosphere, while the Cl^- amount in the throughfall water is directly proportional to the Cl^- amount of open plot precipitation water. The latter fact can only be understood if we suggest that the majority of the Cl^- observed in the samples originates, not from rain, but from dry fallout. Aerosols fall freely into the gauges in an open plot, but accumulate in canopy. Their Cl^- content will only be diluted within the gauges in an open plot but the fallout intercepted in canopy will be washed into the gauges under the canopy by precipitation. This is the reason behind the observed close relationship, although there is more evidence supporting this idea. Figure 6 shows the Cl^- amount for some smaller samplings in the throughfall water versus the Cl^- amount in the open plot precipitation. Data points of samples collected after longer periods are located above the regression line, while those collected after shorter periods are below this line. (Greater precipitation samples contain water from more rainfalls after rainless periods of different duration, so they are average values falling very much closer to the line.)

Some authors also suspect, or even indicate, the importance of dry fallout (BORMANN et al. 1977, WHITE and TURNER 1970, WIMAN 1981). We would like to install two gauges in an open plot, one which is open only during rainfall, and an other which is open only during rainless periods, thus making it possible to distinguish between the input of chemicals with dry fallout and with rainwater.

The average pH value of the rainwater is 5.46, which is in accordance with that of CO_2 saturated water, and excess acidity can be neglected. This slightly acid rainwater is neutralized to a lesser degree by juniper, and to a

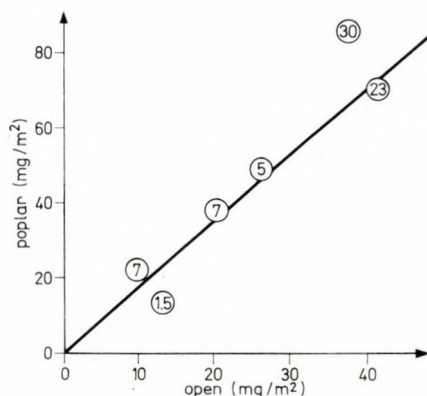


Fig. 6. Collected Cl^- -amounts (mg/m^2) in poplar stand vs. collected Cl^- -amounts (mg/m^2) in open plot. Circles denote observed data. Numbers inside the circles denote time periods between actual and previous samplings. The solid line is the regression line through the origin

greater degree by poplar. The total acid input during the observation period was 30 mg/ha H^+ in the open plot, 8 g/ha in juniper and only 2.5 g/ha in poplar, totals which are of no practical environmental importance.

As to our sampling techniques we concluded that all of the gauges were located on the right places, except one. This was located on the poplar forest boundary, far from the other poplar gauges, and its recordings are often not in accordance with the others. This will be replaced. One of the four open plot gauges significantly collects about 7% less water than the others. This will be changed, although we also suspect similar outlying gauges. Further gauges will be placed in the inner areas of juniper plots.

From our observations, it seems that the optimum periods between subsequent samplings is not more than a fortnight. Furthermore, fresh samplings after individual storms provide valuable information, especially concerning the influence of meteorological factors in water and nutrient input. During subsequent observations, samples collected on a plot (open, juniper or poplar) can be regarded as uniform, especially with respect to their chemical contents. However, we would like to point out that, before deciding so, it is advisable to record the individual data from each gauges and check their homogeneity using statistical methods.

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LICHENS AS INDICATORS OF AIR POLLUTION IN THE BUDAPEST AGGLOMERATION

I. AIR POLLUTION MAP BASED ON FLORISTIC DATA AND HEAVY METAL CONCENTRATION MEASUREMENTS

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The recent distribution of lichens in Budapest was examined and heavy metal concentrations in two lichen species were measured to indicate air pollution zones of Budapest. A lichen zone map was drawn according to the distribution of epiphytic lichen species. This map was compared to a sulphur dioxide zone map, to the distribution map of lichens compiled about 70 years ago and to old and recent maps of the built-up areas. The effect of climate and substrate type upon lichens was also investigated. Because of the absence of epiphytic lichens in the extensive desert zone (downtown Budapest), transplanted *Hypogymnia physodes* and *Cladonia convoluta* samples were taken at 50 points in order to determine the concentration of accumulated heavy metals (Pb, Cd, Mn, Zn).

Introduction

Many investigations have been carried out throughout the world to reveal causes of damage and changed distribution of lichens in cities and big industrial centres. The earliest studies may be dated back to the middle of the last century (GRINDON 1859, NYLANDER 1866). Our studies started in 1979 within the framework of the Environmental Biological Research Program of Budapest Agglomeration elaborated in 1976 (BERCZIK and BORHIDI 1979). Lichen maps of numerous cities and industrial centres have been made in Europe and elsewhere, including data from field and laboratory experiments connected with this problem, e.g., transplantation studies (see reviews by GILBERT 1973, and FERRY, BADDELEY and HAWKSWORTH 1973). In Hungary, however, only early attempts were made in this field: the lichen map of Debrecen was drawn by FELFÖLDY (1942) in a manner described by SERNANDER (1926) and that of Szeged by GALLÉ (1979) who compared his map to sulphur dioxide concentration values measured in the air. In western Hungary KISS (1983) examined lichens as potential indicator plants and emphasized the importance of abnormal succession in lichen assemblages.

In Budapest no such studies have been performed as yet, except a recent transplantation experiment by SOLYMOŠI (1982) whose scattered records (3 sites only) do not supply a comprehensive picture on the air pollution of the capital. Therefore, our main objective is to draw an air pollution map of Budapest on the basis of the presence and abundance of lichens as indicators. In addition, factors influencing the recent lichen distribution were examined by comparing the map to chemical air pollution data and lichen records from the beginning of this century. Finally, the effects of other factors (urban climate, substrate effects, etc.) were also investigated. Since no precise data on the metal contamination of the air were available, a study of heavy metal pollution by lichen transplants was designed.

Portions of this paper are extracted from two M. Sc. theses in biology (FARKAS 1982, Lőkös 1983) prepared under the supervision of the third author. The first author collected the data in the mapping project, while the second author of this paper is responsible for the transplantation experiments and heavy metal concentration measurements.

Brief characterization of the study area

Budapest, the capital of Hungary is situated on the Danube river, at the contact point of the Great Hungarian Plain and the mountains. Its area is 525 km², 352 km² on the left bank and 173 km² on the right. Pest, on the left bank is largely flat at about 100 m a.s.l., whereas Buda on the right bank is hilly (the highest point being 529 m). Relief, climate, natural vegetation of the two parts are different. As a result of urbanization the natural vegetation disappeared turning into a secondary vegetation (ZÓLYOMI 1958). Much of the Buda Hills area is still covered by woodland, although land deterioration by construction work has been rapid.

Materials and methods

I. Mapping

The lichen material (1374 specimens) was collected between October 1979 and March 1982 at 55 points in Budapest (Table 1) and was identified by the first author. In order to find distribution zones the records were indicated in maps at a scale of 1 : 200 000. We prepared map of sulphur dioxide pollution based on available measurements. Maps showing built-up areas and the traffic system were also taken into consideration (Fig. 1).

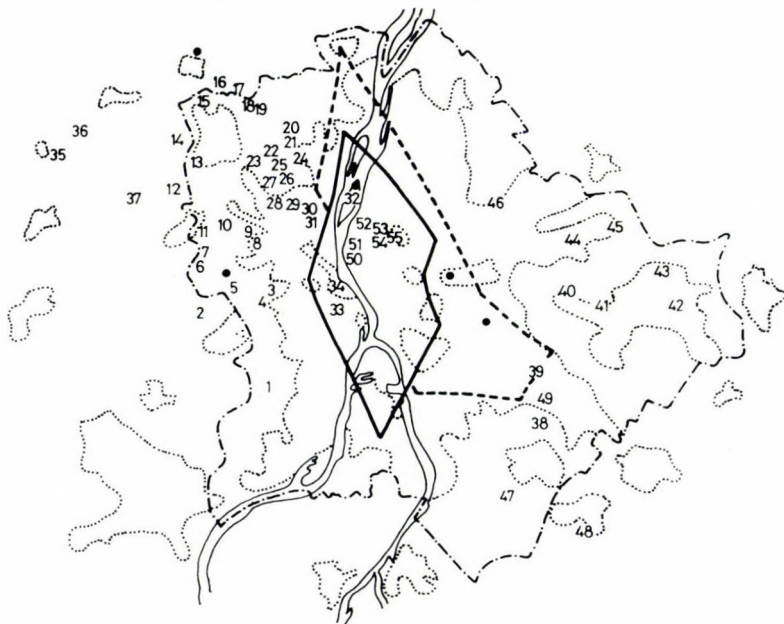


Fig. 1. Fifty-five points of collection, map of sulphur dioxide pollution considered in drawing lichen zones. Symbols and lines indicate sulphur dioxide pollution level in winter: ●: 0–0.09 mg/m³, - - - - - 0.10–0.14 mg/m³, — more than 0.15 mg/m³; boundary of built-up areas, - · - · - · city limits

Table 1
Collecting sites

| Number | Site | Number of collected species | Zone |
|--------|---------------------------------------|-----------------------------|------|
| 1 | Kamara Forest (Tétényi Plateau) | 14 | I |
| 2 | Csíki Hills (Budaörs) | 4 | N |
| 3 | Farkas Valley | 10 | O |
| 4 | Irhás Valley | 5 | O |
| 5 | Csillebérc | 31 | N |
| 6 | Magas-Kő Hill | 7 | N |
| 7 | Makkosmária | 32 | N |
| 8 | Szabadság Hill | 12 | O |
| 9 | Tündér Hill | 22 | O |
| 10 | János Hill | 15 | O |
| 11 | Budakeszi Forest (Budapest) | 7 | N |
| 12 | Vadaspark (Hársbokor Hill, Budakeszi) | 16 | N |
| 13 | Hosszúerdő Hill | 17 | N |
| 14 | Remete Hill | 51 | N |
| 15 | Budaliget | 7 | N |
| 16 | Alsó-Jegenye Valley | 35 | N |
| 17 | Felső-patak Hill | 17 | N |
| 18 | Szarvas Hill | 8 | I |
| 19 | Csúcs Hill | 12 | I |
| 20 | Vihar Hill | 17 | N |
| 21 | Hármashatár Hill | 28 | O |
| 22 | Újlaki Hill | 7 | N |
| 23 | Vadaskert (Hűvösvölgy) | 30 | N |
| 24 | Szépvölgy | 9 | I |
| 25 | Kecske Hill | 3 | N |
| 26 | Látó Hill | 43 | N |
| 27 | Apáthy Cliff | 18 | N |
| 28 | Pasarét | 4 | I |
| 29 | Törökvész | 12 | O |
| 30 | Ferenc Hill (Zöldmál) | 20 | I |
| 31 | Vérhalom | 11 | O |
| 32 | Margaret Island | 3 | I |
| 33 | XI. Kosztolányi D. Square | 2 | I |
| 34 | Gellért Hill | 5 | I |
| 35 | Telki (village) | 1 | N |
| 36 | Telki Hill | 35 | N |
| 37 | Fekete Hills (Budakeszi) | 4 | N |

Table 1 (cont'd)

| Number | Site | Number of collected species | Zone |
|--------|---|-----------------------------|------|
| 38 | XVIII. Pestlőrinc, Halmi Forest | 16 | N |
| 39 | XVIII. Pestlőrinc, Ságvári E. Street | — | I |
| 40 | X. Vadszőlő Street | — | D |
| 41 | XVII. Rákosszabab, Akadémia Újtelep | — | I |
| 42 | XVII. Rákosszabab, Zrínyi Street | — | D |
| 43 | XVII. Rákosszabab, Micsurin Street | — | D |
| 44 | XVI. Mátyásföld, Petőfiker | — | D |
| 45 | XVI. Cinkotai Cemetery | 6 | I |
| 46 | XVI. Sasfalom, Thököly Road | — | D |
| 47 | XX. Péterimajor | — | I |
| 48 | Gyál Railway Station | — | D |
| 49 | XVIII. Pestlőrinc, old cemetery | 3 | I |
| 50 | V. Belgrád Embankment (Lánchíd—Chain Bridge) | — | D |
| 51 | V. Bajcsy-Zsilinszky Road | — | D |
| 52 | VI. Rudas L. Street (Nyugati Railway Station) | — | D |
| 53 | VI. Kodály Circus | — | D |
| 54 | Lövölde Square | — | D |
| 55 | VII. Gorkij Avenue | — | D |

D = desert zone, I = inner struggle zone, O = outer struggle zone, N = normal zone.

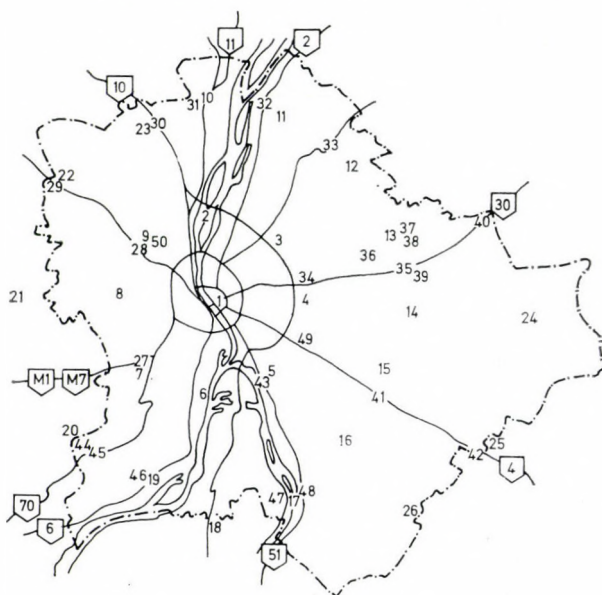


Fig. 2. Fifty sites of transplantation experiments and the most important thoroughfares in Budapest

Table 2
Transplantation sites

| Number | Sites | Description | | | |
|--------|---|---------------------------|-------------------------------------|-----------|--|
| | | Protection from pollution | Distance in metres from the nearest | | Exposition of <i>Hypogymnia physodes</i> samples |
| | | | thoroughfare | side road | |
| 0 | Control | | | | |
| W0 | "Washed" control (soaked in distilled water for 24 hours) | | | | |
| 1 | V. Belgrád Embankment | — | 40 | 1 | s SW |
| 2 | XIII. Margaret Island | + | 60 | 3 | s SE |
| 3 | XIV. Kassai Square | + | 440 | 60 | s NW |
| 4 | X. Pongrác Road × Salgótarjáni Road | + | 1000 | 1 | s NE |
| 5 | IX. Kén Street | — | 60 | 1 | s NW |
| 6 | XI. Árasztó Street | — | 20 | 1 | t SW |
| 7 | XI. Kőérhereki Road × Kétyölgyi Road | + | 250 | 2 | s SE |
| 8/I | XII. Farkasvölgyi Road × Rege Street | — | — | 8 | s NW |
| 8/IIa | The same site as 8/I, but | without washing | | | |
| 8/IIb | different date (20th Oct. 1982) and | washed | | | |
| 8/IIc | exposure time (4 months) | soaked | | | |
| 9 | II. Nagy Lajos Square | + | 300 | 5 | s, t SW |
| 10 | II. Martos Flóra Road × Géza Road | — | 2 | — | t SE |
| 11 | IV. Baross Road × Izzó Road | + | 1100 | 80 | — SW |
| 12 | XV. Mogyoród Road × Páskomliget | + | 1000 | 1000 | s SW |
| 13 | XVI. Hangos Square | + | 1400 | 1 | s NE |
| 14 | Tűzok Street × Rákos Stream | + | 700 | 20 | t S |
| 15 | XVIII. Pestlőrinc, Railway Station | — | 1 | — | t S |
| 16 | XX. Mártírok Road × Erzsébet Square | — | 500 | 400 | s SE |
| 17 | XX. Molnár Island | + | 300 | 20 | — W |
| 18 | XXI. Csepeli Road × 8653rd Street | — | 3 | — | t W |
| 19 | XXII. Nagytétényi Road × Sallai Road | — | 2 | 2 | t NW |
| 20 | Village Diósd, Szabadság Road | + | 1100 | 100 | t S |
| 21 | Village Budakeszi, glider landing place | — | — | 2 | s W |
| 22 | II. Rézsű Street | + | 250 | 2 | s S |
| 23 | III. Solymár Lane, Bp.-Üröm Railway Station | + | 60 | 20 | t NE |
| 24 | XVII. Újtelepi Street | + | 400 | 4 | s NW |
| 25 | Between Férihegy Airport and village Vecsés | + | 800 | 4 | s NW |
| 26 | XVIII. Igric Street | + | 150 | — | s NE |
| 27/I | XI. Budaörsi Street × Highway M7 | — | 3 | — | t SE |
| 27/IIa | The same site as 27/I, but | without washing | | | |
| 27/IIb | different date (20th Oct. 1982) and | washed | | | |
| 27/IIc | exposure time (4 months) | soaked | | | |
| 28 | XII. Szilágyi E. Avenue × Budakeszi Road | — | 1 | — | t NE |
| 29 | II. Nagykovácsi Road, Ady-liget | — | 2 | — | t NE |
| 30 | III. Bécsi Road, Bp.-Üröm Railway St. | — | 2 | — | t NE |
| 31 | III. Hegymászó Road | + | 700 | 30 | t, s SE |
| 32 | IV. Váci Road | — | 3 | — | t NE |
| 33 | XV. Régi Főti Road × Szilas Stream | — | 3 | — | t SE |
| 34 | X. Kerepesi Road × Pillangó Road | — | 1 | — | t N |
| 35 | XVI. Borotvás Street × Veres Péter Street | — | 2 | — | t NW |
| 36 | XVI. Havashalom Street × Jenőhalom Street | + | 800 | 3 | s NE |

Table 2 (cont'd)

| Number | Sites | Protection from pollution | Description | | Exposition of <i>Hypogymnia physodes</i> samples | |
|--------|--|---------------------------|-------------------------------------|-----------|--|----|
| | | | Distance in metres from the nearest | | | |
| | | | thoroughfare | side road | | |
| 37 | XVI. Ida Street × Csallóközi Street | + | 1600 | 100 | s | NW |
| 38 | XVI. Ida Street × Sarkad Street | + | 1500 | 30 | — | — |
| 39/O | XVI. Kolozsvári Street | + | 700 | 1 | s | SW |
| 39/I | XVI. Kolozsvári Street | + | 700 | 1 | — | NE |
| 40 | XVI. Szabadföld Road × Akácos Road | — | 2 | — | t | SW |
| 41 | XVIII. Vöröshadsereg Road × Mikszáth K. Road | — | 2 | — | t | SW |
| 42 | XVIII. Steinmetz Monument | — | 3 | — | t | SW |
| 43 | IX. Soroksári Road × Illatos Road | — | 60 | — | t | NE |
| 44/O | XXII. Szakiskola Street × Balatoni Street | + | 15 | — | t | SE |
| 44/I | XXII. Szakiskola Street × Balatoni Street | + | 15 | — | — | NW |
| 45 | XXII. Balatoni Road | — | 2 | — | t | S |
| 46 | XXII. 888th Street × Mátra Street | + | 100 | 20 | t | S |
| 47 | XXI. Hollandi Road × Kormányos Road | + | 500 | 5 | t, s | NE |
| 48 | XX. Marx Károly Road | — | 2 | — | t | NE |
| 49 | X. Üllői Road × Bihari Road | — | 10 | 20 | t | SW |
| 50 | II. Endrődi Sándor Street | + | 900 | 30 | t, s | SW |

× = crossing, s = towards side road, t = towards thoroughfare, O = on the outer side of the tree (towards the street), I = on the inner side of the tree.

II. Transplantation

Hypogymnia physodes (L.) Nyl., an epiphytic species, and *Cladonia convoluta* (Lam.) P. Cout., an epigeic lichen, were used in the transplantation experiments. The material was collected in a control area 40 km SE of Budapest supposed to be free of immission. Transplanted *H. physodes* samples consisted of about 6–8 thalli sewn on a piece of felt (8 × 8 cm²). *Cl. convoluta* samples were placed on little boxes filled with sand and were sewn on, too. They were transplanted at 50 points of Budapest (Fig. 2, Table 2).

III. Determination of heavy metal content

After 3 months exposure time digestion was carried out in 10 cm³ of ccHNO₃ and immediately in 1.5 cm³ of 30% H₂O₂ using 1 g air dried lichen material. Heavy metal contents (Pb, Cd, Mn, Zn) were measured by Pye Unicam SP 9 atomic absorption spectrophotometer.

Results and discussion

I. The air pollution zones of Budapest established on the basis of epiphytic lichen distribution

Four different zones of lichen distribution were recognized (Fig. 3). Species occurring in an inner zone are also present in all outer zones. The species of the area are listed by VERSEGHY and FARKAS (1984).

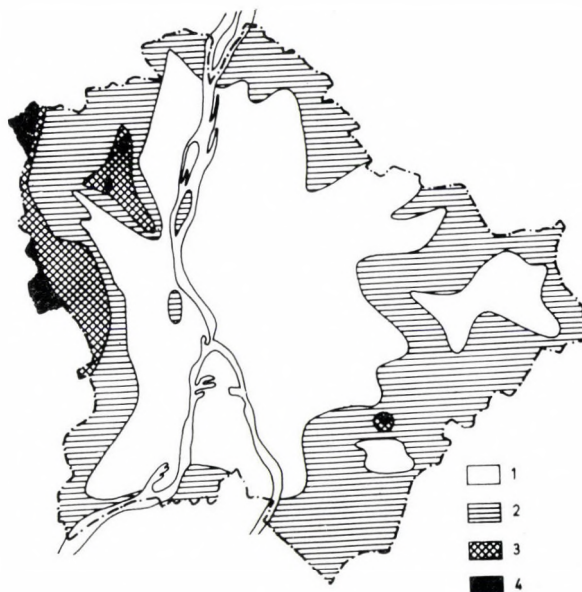


Fig. 3. Air pollution zones of Budapest based on the distribution of epiphytic lichens (1 = desert zone, 2 = inner struggle zone, 3 = outer struggle zone, 4 = normal zone)

1. Desert zone

The built-up area of Budapest is in fact a lichen desert without any lichens. The boundary of this zone corresponds approximately to the boundary of built-up areas. There was only a small portion of the built-up area, northern Buda, in which lichens were found. Sulphur dioxide concentration is more than 0.10 mg/m^3 (even 0.30 mg/m^3 in winter) in this zone.

2. Struggle zones

Two struggle zones are situated in the outer areas of Budapest. Lichens are present but only a few characteristic species occur. The most resistant crustose epiphytic lichen species, *Scoliciosporum chlorococcum* (Stenh.) Vězda, *Lecanora conizaeoides* Nyl. ex Crombie, *L. varia* (Hoffm.) Ach. and *Buellia punctata* (Hoffm.) Mass. are recorded in both struggle zones. We found only little thalli ($1\text{--}2 \text{ cm}^2$) of the foliose lichens, *Hypogymnia physodes* (L.) Nyl. and *Parmelia sulcata* Tayl.

(a) Inner struggle zone

This zone surrounds the built-up areas of Budapest and forms some patches within the desert zone (at points 32 and 33–34, see Table 1) as well. This zone extending to the boundary of Pest can be characterized by the occurrence of *Lecanora piniperda* Koerb. and *L. hageni* (Ach.) Ach.

(b) Outer struggle zone

This is transitional between the inner struggle zone and the normal zone, and is mainly represented by the higher and outer regions of Buda. The boundary of this zone is drawn according to the presence of *Lecanora sarcopis* (Ach.) Ach., *L. subintricata* (Nyl.) Th. Fr. and *Lecidea lucida* (Ach.) Ach.

3. Normal zone

This is the zone where more or less natural lichen flora is found. Its area is restricted to a small part of Buda near the western boundary of Budapest. The boundary of normal zone is determined by the presence of *Parmelia glabrata* (Lamy) Nyl. var. *fuliginosa* (Fr. ex Duby) Grumm.

II. Factors influencing the recent distribution of lichens

1. Urbanization

Urbanization is the process of concentrating population and industrial plants in big cities. In Budapest this process was described by PREISICH (1969).

An intensive development started in 1867 (after the Austro-Hungarian Compromise). While Pest, from the Middle Ages through the modern times has been an industrial and commercial centre, Buda was a fortified royal seat, and a town of strategic importance. Óbuda, to the north of it, was a market town with an agricultural character. These three towns were independent and separate from one another until they were merged as the city of Budapest in 1873. Our capital had become a metropolis by the time of the First World War.

Now it has more than 2 million inhabitants (one fifth of the population of Hungary). In addition, Budapest is a considerable industrial centre with a traffic system steadily increasing. The downtown surrounded formerly by walls lies directly on the Danube. The street pattern resembles to a spider's web.

The buildings occupy large, originally wooded areas, so there are in the city much less trees as potential substrate for lichens. Air pollution is also a result of urbanization. Air pollutants have been derived from the heating of apartment buildings, the operating of industrial plants and nowadays more and more from the traffic (VÁRKONYI 1982).

The earliest lichen records we found in the literature are reported from the beginning of this century. SÁNTHA (1910) and TIMKÓ [(1915) 1925] described the lichen flora of Buda Hills only. Their data (species number) (Fig. 4) compared to the recent data (Fig. 5) show the area "inhabited" by lichens to be decreased considerably. On both maps the lichen-inhabited area extends to the boundary of built-up areas. As seen, considerable area with natural lichen vegetation at the beginning of the century has become a lichen desert by now.

2. Air pollution

(a) General picture of air pollution in Budapest

Air pollution in Budapest as compared with other metropolises is of average degree (VÁRKONYI 1982).

In the nineteenth century dust was the most important air pollutant in Hungary. First, FODOR (1879, cited in PROBÁLD 1974) examined dust and carbon monoxide pollution in Budapest. KOGUTOWICZ (1913) prepared the first air pollution map of Budapest taking sources of pollution and direction of wind into consideration. From the 1930s sulphur compounds came into prominence to be responsible for air pollution (SCHEFF and DABIS 1932, WALDBAUER 1938, MÓRIK 1961, 1967, FEHÉR 1970, ÁRVAI 1971).

Nowadays lead, carbon monoxide and nitrogen monoxide pollution deriving from the traffic tends to become more and more severe (VÁRKONYI 1982). These are more dangerous than the pollutants deriving from heating because lead, carbon monoxide and nitrogen monoxide get to the atmosphere close to the ground and immediately are inhaled by man.

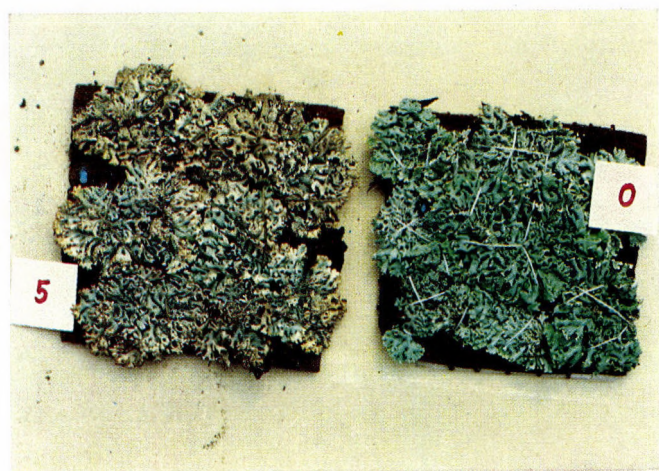


Fig. 6. Damaging effects of the air on the *Hypogymnia physodes* sample of site 5 near a sulphuric acid factory. Compare with the control (0)

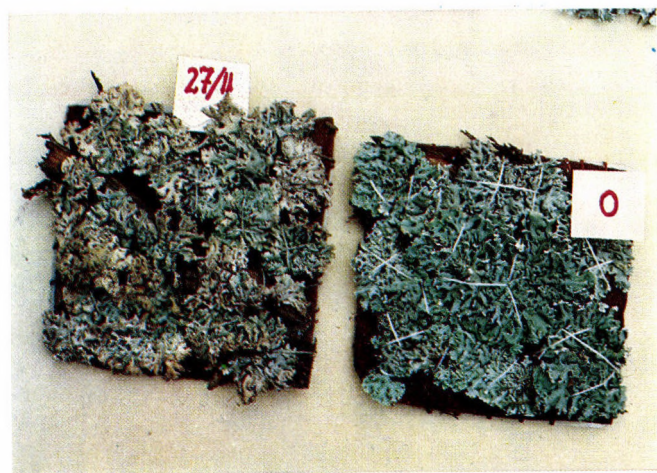


Fig. 7. Damaged *Hypogymnia physodes* sample from site 27/II near a thoroughfare. Compare with the control (0)



Fig. 8. Damaged *Hypogymnia physodes* sample from site 7 near a side street. Compare with the control (0)

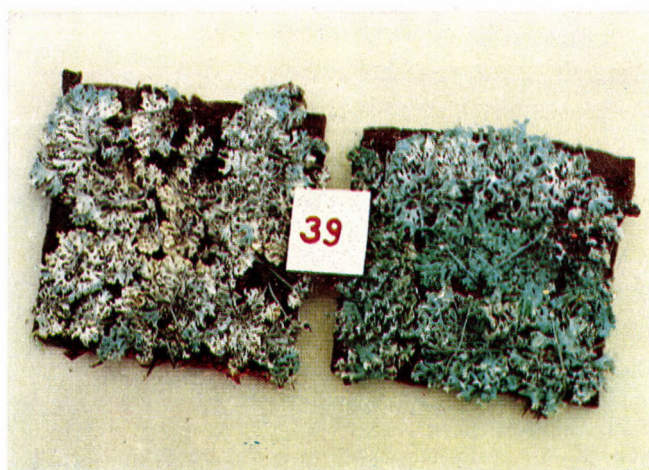


Fig. 9. *Hypogymnia physodes* samples transplanted to two sides of a tree: near the street (left) and on the opposite side (right)

Industrial pollutants on the one hand are heating derivatives, i.e., sulphur and carbon compounds and soot and, on the other hand, are heavy metals (e.g., Zn, Cd, Cu, Mn, Cr, Fe, etc.) and organic compounds and solvents.

The most important reason for the impoverishment of the lichen flora in Budapest may be air pollution including sulphur dioxide pollution originating from urbanization and lead and other heavy metal pollution deriving from increased traffic and industrial activity.

(b) Joint influence of pollutants on the exterior of lichens

The effect of each pollutant mentioned in the previous paragraph is severe by itself but the individual effects cannot be distinguished in a field experiment, like transplantation when the pollutants affect jointly. The sum of the individual effects is not identical with the joint effect because of the interaction of pollutants.

As a result of the air pollution of Budapest different external transformations took place on thalli of *H. physodes* and *Cl. convoluta* (Figs 6–9).

Lichens transplanted to point 5 (cf. Table 2) show the damaging effects of air near a sulphuric acid factory (Fig. 6). The margin of lobes is yellowish or reddish, in contrast with the control. Discolouration may be explained by dehydration or oxidation of some lichen substance, since lichen samples heated to 105 °C were also observed to become reddish.

Damage of chlorophyll in lichen-photobionts caused by air pollutants is seen in specimens which lost their colour. LEBLANC and RAO (1973), IKONEN and KÄRENlampi (1976), KAUPPI and KAUPPI (1976, 1978) accuse sulphur dioxide of chlorophyll degradation and discolouration but lead also can cause it since lead prevents chlorophyll synthesis and this also leads to discolouration (RAO et al. 1977). Lichens transplanted close to a main road suffered more serious damages than lichens near a quiet street (Figs 7 and 8).

Of the *H. physodes* samples transplanted to two sides of a tree, those near the street were more severely damaged than the samples on the opposite side (Fig. 9).

(c) Sulphur dioxide pollution maps and the lichen map

Sulphur compounds have an important role among pollutants. Sulphur dioxide is thought to be the indicator of urban air pollution (MÓRIK 1967, FEHÉR 1970, ÁRVAI 1971).

On the basis of maps (Figs 10 and 11) of total sulphur pollution (S mg/100 hour) measured by the Liesegang-method in the heating periods and in the summers (May–October) of 1963–68 PROBÁLD (1974) concluded that pollution was higher in winter when pollutants derived mainly from heating of flats and from industry. The most polluted areas are the town-centre and industrial areas in districts XIII and IX, and also in Csepel (district XXI) in the south. In the summer map the most polluted areas are almost identical with the former ones, but the centre is loaded with less sulphur and each pollution level is lower than in winter. Pollution derives mainly from industry in this case. On the summer map the highly polluted area separated on the north corresponds to an industrial centre of district XIII and the bigger patch shows all other industrial centres.

On the basis of sulphur dioxide concentrations (Table 3) measured by the Institute for Public Health and Epidemiology of the City of Budapest a sulphur dioxide zone map was made (see Fig. 1) which strongly correlates with the lichen distribution. Permitted concentration of sulphur dioxide in protected areas is 0.15 mg/m³. The centre represents a zone with sulphur dioxide concentration higher than 0.15 mg/m³. The next lower polluted zone (between 0.10 and 0.14 mg SO₂/m³) extends to the southeast because of the northwestern prevailing winds and the situation of built-up areas. Lower values are too scarce to recognize further zones.

Lichens have disappeared from both polluted areas. While in Buda under $0.10 \text{ mg SO}_2/\text{m}^3$ lichens (even *P. glabratula* var. *fuliginosa*, a foliose lichen) occur, in Pest they are absent from these low polluted areas. That is why we must investigate effects of other polluting factors, mostly exhaust gas pollution, and climate and substrate type.



Fig. 10. Total sulphur pollution (S mg/100 hour) in winter (PROBÁLD 1974)

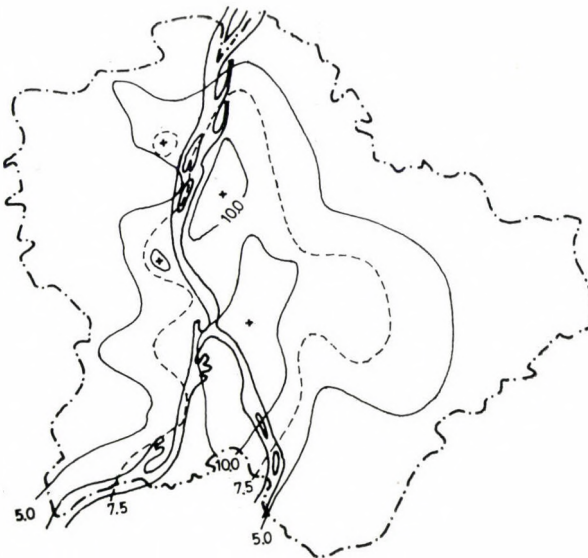


Fig. 11. Total sulphur pollution (S mg/100 hour) in summer (PROBÁLD 1974)

Table 3
*Sulphur dioxide concentrations (mg/m^3) in the air
of Budapest in 1975*

| Site No. | Winter monthly average (October– March) | January | Summer monthly average (April– November) | July |
|----------|--|---------|---|------|
| 1 | 0.19 | 0.30 | 0.03 | 0.01 |
| 2 | 0.30 | 0.33 | 0.07 | — |
| 3 | 0.21 | 0.21 | 0.03 | 0.01 |
| 4 | 0.11 | 0.18 | 0.02 | 0.01 |
| 5 | 0.12 | 0.21 | 0.01 | 0.00 |
| 6 | 0.15 | 0.25 | 0.03 | 0.01 |
| 7 | 0.11 | 0.22 | 0.02 | 0.01 |
| 10 | 0.26 | 0.40 | 0.04 | 0.02 |
| 11 | 0.17 | — | 0.02 | 0.01 |
| 13 | 0.23 | — | 0.04 | 0.03 |
| 17 | 0.19 | 0.33 | 0.04 | 0.02 |
| 18 | 0.16 | — | 0.05 | 0.05 |
| 19 | 0.17 | 0.23 | 0.04 | 0.02 |
| 20 | 0.16 | 0.35 | 0.03 | 0.01 |
| 21 | 0.17 | 0.26 | 0.04 | 0.02 |
| 22 | 0.09 | — | 0.03 | 0.00 |
| 23 | 0.12 | — | 0.03 | 0.02 |
| 24 | 0.09 | — | 0.04 | 0.03 |
| 25 | 0.13 | — | 0.04 | 0.02 |
| 31 | 0.08 | 0.11 | 0.02 | 0.01 |
| 32 | 0.28 | 0.29 | 0.02 | 0.01 |
| 33 | 0.07 | — | 0.03 | 0.02 |
| 34 | 0.17 | 0.28 | 0.05 | 0.03 |
| 35 | 0.16 | 0.36 | 0.03 | 0.00 |
| 39 | 0.18 | — | 0.05 | 0.03 |
| 40 | 0.11 | — | 0.03 | 0.01 |
| 48 | 0.11 | 0.16 | 0.03 | 0.01 |
| 49 | 0.14 | 0.26 | 0.05 | 0.04 |
| 51 | 0.11 | — | 0.02 | 0.01 |
| 52 | 0.12 | — | 0.03 | 0.02 |
| 53 | 0.21 | 0.25 | 0.04 | 0.02 |
| 54 | 0.16 | 0.29 | 0.04 | 0.04 |
| 56 | 0.08 | 0.15 | 0.01 | 0.01 |

Unpublished data obtained from the Institute for Public Health and Epidemiology of the City of Budapest.

Table 4

Heavy metal concentrations in transplanted lichen samples
($\mu\text{g/g}$)

| Number | <i>Hypogymnia physodes</i> | | | | | | | | <i>Cladonia convoluta</i> | | | | | | | |
|--------|----------------------------|-----|-----|-----|----|----|-----|----|---------------------------|----|-----|-----|-----|----|-----|---|
| | Pb | | Cd | | Mn | | Zn | | Pb | | Cd | | Mn | | Zn | |
| | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| 0 | 54 | | 1.6 | | 22 | | 168 | | 67 | | 1.0 | | 31 | | 100 | |
| W0 | 47 | | 1.5 | | 21 | | | | 60 | | 1.0 | | 24 | | | |
| 1 | 171 | 117 | 2.6 | 1.0 | 70 | 48 | — | — | — | — | — | — | — | — | — | — |
| 2 | 68 | 14 | — | — | 32 | 10 | — | — | — | — | — | — | — | — | — | — |
| 3 | 90 | 36 | 1.7 | 0.1 | 64 | 42 | — | — | — | — | — | — | — | — | — | — |
| 4 | 185 | 131 | 2.4 | 0.8 | 98 | 76 | — | — | — | — | — | — | — | — | — | — |
| 5 | 95 | 41 | 2.1 | 0.5 | 35 | 13 | — | — | — | — | — | — | — | — | — | — |
| 6 | 87 | 33 | 2.3 | 0.7 | 42 | 20 | — | — | — | — | — | — | — | — | — | — |
| 7 | 69 | 15 | 1.5 | | 34 | 12 | — | — | — | — | — | — | — | — | — | — |
| 8/I | 53 | | 2.7 | 1.1 | 39 | 17 | — | — | — | — | — | — | — | — | — | — |
| 8/IIa | 71 | 17 | 1.6 | 0.0 | 32 | 10 | — | — | — | — | — | — | — | — | — | — |
| 8/IIb | 64 | 10 | 1.5 | | 30 | 8 | — | — | — | — | — | — | — | — | — | — |
| 8/IIc | 60 | 6 | 1.9 | 0.3 | 35 | 13 | — | — | — | — | — | — | — | — | — | — |
| 9 | 68 | 14 | 1.8 | 0.2 | 28 | 6 | — | — | — | — | — | — | — | — | — | — |
| 10 | 63 | 9 | 1.8 | 0.2 | 45 | 23 | — | — | — | — | — | — | — | — | — | — |
| 11 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 12 | 72 | 18 | 1.7 | 0.1 | 43 | 21 | — | — | — | — | — | — | — | — | — | — |
| 13 | 95 | 41 | 1.8 | 0.2 | 80 | 58 | — | — | — | — | — | — | 120 | 89 | — | — |
| 14 | 103 | 49 | 3.5 | 1.9 | 48 | 26 | — | — | — | — | — | — | — | — | — | — |
| 15 | 153 | 99 | 2.3 | 0.7 | 21 | | — | — | — | — | — | — | — | — | — | — |
| 16 | 49 | | 1.9 | 0.3 | 48 | 26 | — | — | — | — | — | — | — | — | — | — |
| 17 | 76 | 22 | 1.8 | 0.2 | 50 | 28 | — | — | — | — | — | — | — | — | — | — |
| 18 | 136 | 82 | 2.2 | 0.6 | 60 | 38 | — | — | 151 | 84 | 1.4 | 0.4 | 109 | 78 | — | — |
| 19 | 92 | 38 | 2.5 | 0.9 | 55 | 33 | — | — | 131 | 64 | 2.8 | 1.8 | — | — | — | — |
| 20 | 49 | | 2.3 | 0.7 | 44 | 22 | — | — | — | — | — | — | — | — | — | — |
| 21 | 75 | 21 | 1.8 | 0.2 | 34 | 12 | — | — | — | — | — | — | — | — | — | — |
| 22 | 64 | 10 | 2.1 | 0.5 | 43 | 21 | — | — | — | — | — | — | — | — | — | — |
| 23 | 56 | 2 | 1.9 | 0.3 | 42 | 20 | — | — | — | — | — | — | — | — | — | — |
| 24 | 68 | 14 | — | — | 48 | 26 | — | — | — | — | — | — | — | — | — | — |
| 25 | 93 | 39 | 1.8 | 0.2 | 53 | 31 | — | — | — | — | — | — | — | — | — | — |
| 26 | 78 | 24 | 1.7 | 0.1 | 48 | 26 | 212 | 44 | — | — | — | — | — | — | — | — |
| 27/I | 134 | 80 | 1.9 | 0.3 | 30 | 8 | — | — | — | — | — | — | — | — | — | — |
| 27/IIa | 174 | 120 | 2.5 | 0.9 | 63 | 41 | — | — | — | — | — | — | — | — | — | — |
| 27/IIb | 172 | 118 | — | — | 49 | 27 | — | — | — | — | — | — | — | — | — | — |
| 27/IIc | 171 | 117 | 3.1 | 1.5 | 49 | 27 | — | — | — | — | — | — | — | — | — | — |

Table 4 (cont'd)

| Number | <i>Hypogymnia physodes</i> | | | | | | | | <i>Cladonia convoluta</i> | | | | | | | |
|--------|----------------------------|-----|-----|-----|-----|-----|-----|----|---------------------------|-----|-----|-----|-----|-----|-----|-----|
| | Pb | | Cd | | Mn | | Zn | | Pb | | Cd | | Mn | | Zn | |
| | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| 28 | 77 | 23 | 1.6 | 0.0 | 41 | 19 | — | — | 132 | 65 | 1.3 | 0.3 | 69 | 38 | — | — |
| 29 | 71 | 17 | 1.5 | | 46 | 24 | — | — | — | — | — | — | — | — | — | — |
| 30 | 131 | 77 | 1.8 | 0.2 | 56 | 34 | 196 | 28 | — | — | — | — | — | — | — | — |
| 31 | 59 | 5 | 1.9 | 0.3 | 27 | 5 | — | — | — | — | — | — | — | — | — | — |
| 32 | 102 | 48 | 1.8 | 0.2 | 52 | 30 | — | — | 117 | 50 | 2.8 | 1.8 | 133 | 102 | — | — |
| 33 | 298 | 244 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 34 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 35 | 325 | 271 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 36 | 69 | 15 | 1.9 | 0.3 | 39 | 17 | — | — | — | — | — | — | — | — | — | — |
| 37 | 112 | 58 | 3.3 | 1.7 | 58 | 36 | 187 | 19 | — | — | — | — | — | — | — | — |
| 38 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 39/O | 81 | 27 | 1.8 | 0.2 | 21 | | — | — | — | — | — | — | — | — | — | — |
| 39/I | 68 | 14 | 1.7 | 0.1 | 53 | 31 | — | — | — | — | — | — | — | — | — | — |
| 40 | 115 | 61 | 4.1 | 2.5 | 71 | 49 | 193 | 25 | — | — | — | — | — | — | — | — |
| 41 | 75 | 21 | 5.2 | 3.6 | 43 | 21 | — | — | — | — | — | — | — | — | — | — |
| 42 | 131 | 77 | — | — | 65 | 43 | 219 | 51 | — | — | — | — | — | — | — | — |
| 43 | 78 | 24 | 1.6 | 0.0 | 133 | 111 | — | — | 200 | 123 | 2.1 | 1.1 | — | — | — | — |
| 44/O | 102 | 48 | 2.6 | 1.0 | 55 | 33 | — | — | — | — | — | — | — | — | — | — |
| 44/I | 76 | 22 | 2.1 | 0.5 | 58 | 36 | — | — | — | — | — | — | — | — | — | — |
| 45 | 107 | 53 | 1.6 | 0.0 | 46 | 24 | — | — | 371 | 304 | 1.1 | 0.1 | 135 | 104 | 219 | 119 |
| 46 | 75 | 21 | 1.9 | 0.3 | 32 | 10 | — | — | — | — | — | — | — | — | — | — |
| 47 | 67 | 13 | 1.8 | 0.2 | 47 | 25 | — | — | 119 | 52 | 1.9 | 0.9 | 107 | 76 | — | — |
| 48 | 109 | 55 | 1.5 | | 50 | 28 | — | — | — | — | — | — | — | — | — | — |
| 49 | 93 | 39 | 1.8 | 0.2 | 132 | 110 | — | — | 167 | 100 | 1.7 | 0.7 | 63 | 32 | — | — |
| 50 | 71 | 17 | 3.4 | 1.8 | 42 | 20 | — | — | — | — | — | — | — | — | — | — |

a = actual measurement, b = a - a₀ = difference from the control.

(d) *Lead and other heavy metals accumulated in lichens and their relation to the lichen map*

Measured concentrations of heavy metals are found in Table 4. Values in column "a" are actual values directly measured in thalli, values in column "b" are actual accumulated amounts (difference between actual and control values).

Heavy metal content in *H. physodes* samples

Lead

Lead values vary between 0 and 271 µg/g (Fig. 12). The control value (54 µg/g) approximates that measured by DERUELLE and PETIT (1983 — 65 µg/g) in France and is higher than other values in the literature (10 µg/g — PAKARINEN and MÄKINEN 1976, 35 µg/g — MÄKINEN

and PAKARINEN 1977, 15 $\mu\text{g/g}$ — TAKALA and OLKKONEN 1981) because our area thought to be free of immission has actually been polluted at a very low level.

There was only a slight difference between washed and unwashed samples in case of samples 8 and 27. The treatments were either fast washing with distilled water or soaking in distilled water for 24 hours.

Values in the samples placed facing to the road were higher than those in the samples on the opposite side of the tree.

Samples near roads (10, 19, 27, 28, 29, 30, 35, 42, 45, 48) accumulated more lead than those taken far apart (7, 9, 13, 17, 20, 22, 23, 25, 31, 36, 37, 39, 44, 46, 47) in every case (Fig. 13) similarly to the measurements of BURKITT et al. (1972), SMITH (1976), RAO et al. (1977), TAKALA and OLKKONEN (1981) though their values are higher than ours. A possible interpretation is that our continental climate rarely supplies sufficient amount of moisture for lichen growth and so they can accumulate pollutants for shorter time in contrast with those of, for instance, the Scandinavian countries. Lower values measured farther from roads are due to the different protective covers (e.g., buildings, rows of trees, bushes or else that are barriers of air pollution). Different protective covers result in differences in measurements.

Along some transects through the city lead concentration increases towards the centre (Fig. 14). Values for North-Buda are lower than those indicated in Pest. These are in accordance with the lichen distribution.

Cadmium

Cadmium concentration varies between 0.1 and 3.6 $\mu\text{g/g}$ (Fig. 15). The control value, 1.6 $\mu\text{g/g}$, is too high if compared to that obtained by PILEGAARD (1979 — 0.58 $\mu\text{g/g}$). The control value only slightly differs from the washed control (1.5 $\mu\text{g/g}$).

Cadmium concentrations probably do not relate to the traffic because some values were higher for N-Buda, far from main roads, where lead contents are low, and elsewhere low cadmium content but high lead values were obtained, especially near main roads. The two highest values were obtained at two main roads in Budapest.

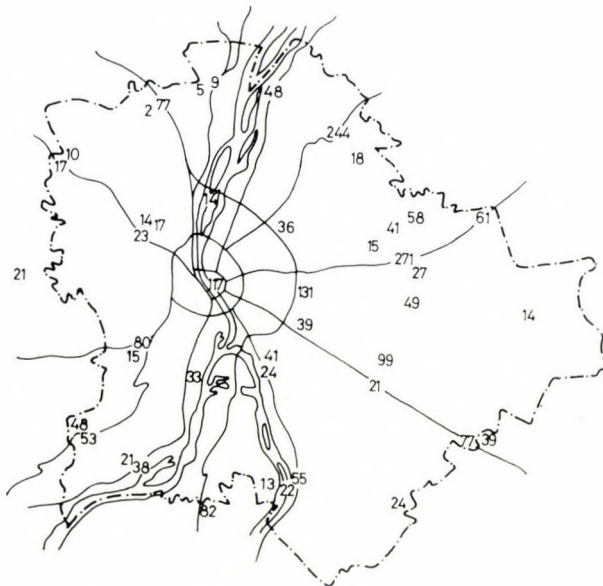


Fig. 12. Lead accumulation ($\mu\text{g/g}$) in *Hypogymnia physodes* transplanted samples

It is conceivable that cadmium pollution is of industrial origin, or there is a negative interaction between lead and cadmium accumulation.

Manganese

Values of manganese content vary between 5 and 111 $\mu\text{g/g}$ (Fig. 16). Our control value (22 $\mu\text{g/g}$) almost equals the one obtained by PILEGAARD (1979 — 24 $\mu\text{g/g}$). The control value is 1 $\mu\text{g/g}$ higher than the washed control value (21 $\mu\text{g/g}$).

The manganese pollution of Buda is less than that in Pest. Values higher than 50 $\mu\text{g/g}$ occur only in Pest.

The dependence upon the closeness of roads is higher than in the case of cadmium (compare sample pairs 10–31, 29–22, 19–46, 42–25, 30–23, 28–9, 43–5), but it is supposed that manganese pollution derives rather from the steel parts of engines than from the fuel (like lead).

Zinc

Our possibilities were limited in case of zinc contents. The values measured provide only preliminary information.

The values vary between 17 and 51 $\mu\text{g/g}$. The control value (168 $\mu\text{g/g}$) is close to the value (160 $\mu\text{g/g}$) measured by LAAKSOVIRTA and OLKKONEN (1979) and is twice as high as that given by PILEGAARD (1979 — 85 $\mu\text{g/g}$).

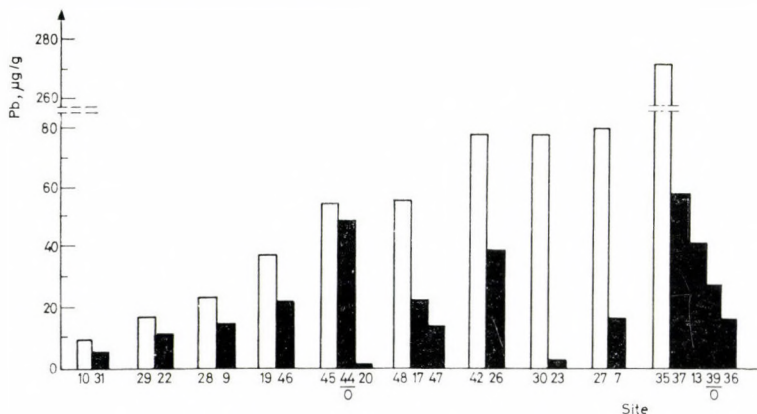


Fig. 13. Lead content of *Hypogymnia physodes* samples near thoroughfares (white) and side streets (black)

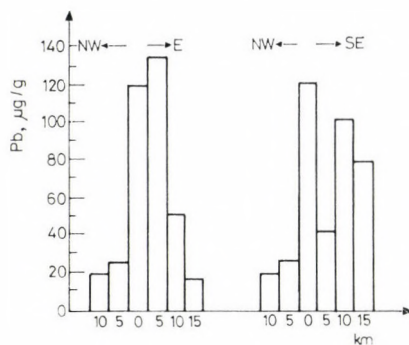


Fig. 14. Change of lead content of *Hypogymnia physodes* samples in two different directions. Numbers indicate distance from the city centre

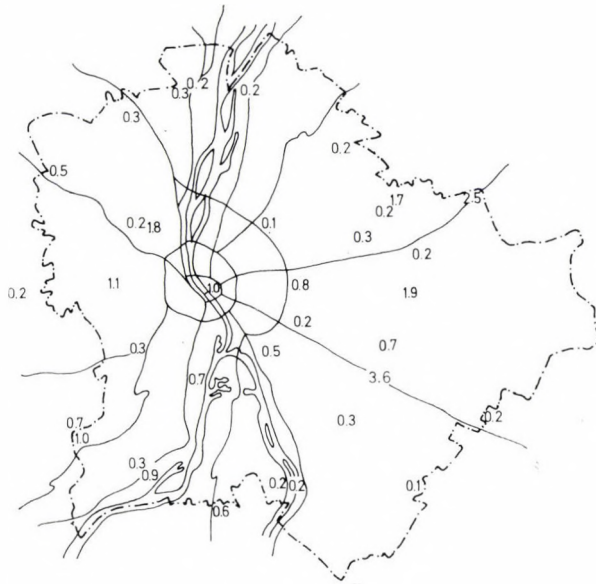


Fig. 15. Cadmium accumulation ($\mu\text{g/g}$) in *Hypogymnia physodes* transplanted samples

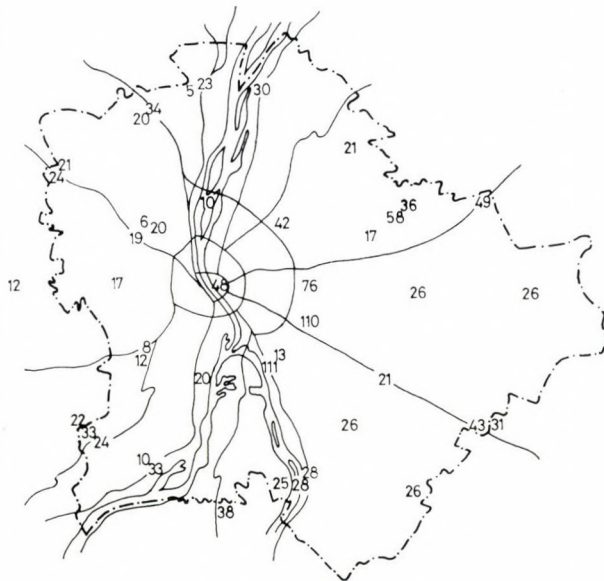


Fig. 16. Manganese accumulation ($\mu\text{g/g}$) in *Hypogymnia physodes* transplanted samples

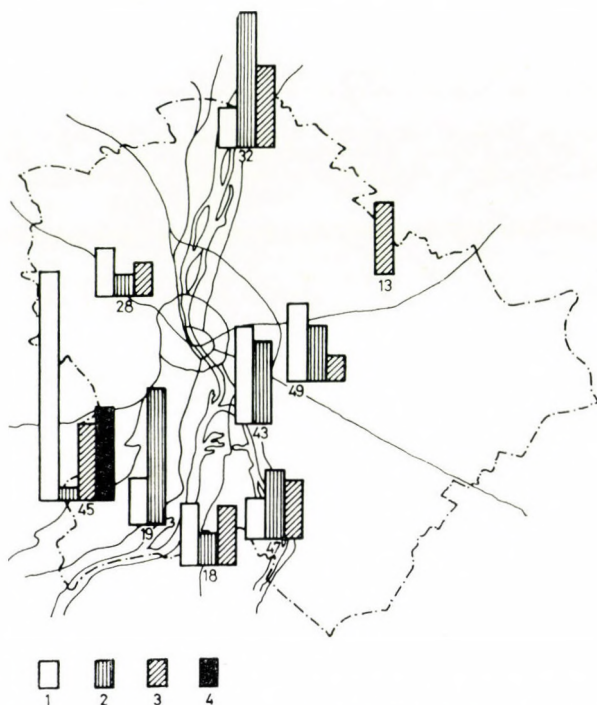


Fig. 17. Heavy metal accumulation in *Cladonia convoluta* transplanted samples. Concentrations relate to dry matter. One unit in the histogram corresponds to 50 $\mu\text{g/g}$ for lead (1), manganese (3), and zinc (4), and 0.5 $\mu\text{g/g}$ for cadmium (2)

Cladonia convoluta

Only few measurements in *Cl. convoluta* are available because several transplanted samples were not found in the site.

Values measured are: lead — 50–304 $\mu\text{g/g}$, cadmium — 0.1–1.8 $\mu\text{g/g}$, manganese — 32–104 $\mu\text{g/g}$, zinc — 119 $\mu\text{g/g}$ (Fig. 17). All values are higher than those for *H. physodes*. *Cl. convoluta* accumulates much more heavy metals than *H. physodes*. This fact is in accordance with the observation by KAUPPI (1976) whereas epigeic fruticose lichens are more suitable for short-term indication than the corticolous species. Since *Cl. convoluta* is a xerophyllous species, it is suitable for indication of air pollution under the urban circumstances in Hungary.

3. Urban climate

Today the climate of Budapest is a special anthropogenous mesoclimate, namely urban climate (PROBÁLD 1974). The climates of the two parts of Budapest were originally different, just like the natural vegetation. These differences can be seen in the changed urban climate, too.

Limiting climatic factors of natural distribution of lichens are humidity, precipitation, temperature and wind. The change of these factors affects lichens unfavourably.

The difference between the annual mean of relative humidity (expressed as percentage) in Kitaibel Pál street, Buda and in Pestlőrinc, Pest is about 3.9. Relative humidity is lower in cities than around them because of higher temperature and the lack of natural evaporating

surface. Despite this fact, the precipitation in cities is more because of air pollution (condensation cores, etc.) but greater amount of precipitation flows away immediately through drain pipes and the residue evaporates quickly as a result of the higher temperature.

Buda Hills have more precipitation (675 mm) than downtown Pest (525 mm). At the same time the mean temperature is about 2 °C higher in the centre than in the surroundings. The city centre is more arid than the driest and most droughty areas of the country in terms of BORHIDI's index of semiaridity and in climatic water deficiency (BERCZIK and BORHIDI 1979).

After preparing WALTER's climatic diagrams for 6 points in Budapest, the climate of Madách Square in the city centre turned out to be the most arid and that of Szabadság Hill in the Buda Hills to be the most humid (see Fig. 5 in VERSEGHY and FARKAS 1984). The semiarid period is short (August–September) in Szabadság Hill and longer (from July to October) at the other points. During this period lichens are almost entirely inactive, they assimilate only if some moisture is available. The air pollution level is low (0–0.07 mg SO₂/m³) as compared to the winter months (0–0.40 mg/m³), when lichens are active.

Urban wind system makes lichens drier and spreads air pollutants over the whole capital. The distribution of lichens in Budapest corresponds to the direction of prevailing winds (NW). In the northwestern part of Budapest lichens occur even in the built-up area, while towards SE lichens gradually disappear from trees and stones. The more polluted area shown in the sulphur dioxide pollution map of Budapest extends also to the southeast.

According to VÁRKONYI (1982) accumulation of pollutants may be increased by some climatic factors, e.g., high relative humidity (which is favourable for lichens).

The urban climate, although not the only limiting factor, can considerably influence the distribution of lichens.

4. Substrate effects

Examining the effects of urbanization we concluded that in addition to increasing air pollution and climatic changes, the large built-up areas are responsible for the disappearance of the natural vegetation. Decrease in forested and wooded areas implies a decrease in available substrate appropriate for epiphytic lichens.

In collecting lichens interest was focussed upon the epiphytic species, because their sensitivity to air pollution is higher than that of epilithic and epigeic lichens (BRODO 1966, SKYE 1968, GALLÉ 1979). The lichen assemblages on trunks are influenced by three important factors (FELFÖLDY 1941):

- the age of the tree,
- the quality of the bark,
- the habitat of the tree (microclimatic effects).

Of course, the lichen assemblages of young and old trees are different. The difference may be accounted mainly for the physical state of the bark (smoothness, chasms, scaling, hardness) which is affected by age. In some places (e.g., points 32 and 38 in Table 1) we found lichens only on the bark of old trees (of about 3–4 m trunk circumference) or on fallen, decayed trees, but we did not find lichens on young trees at the same site.

The quality of the bark varies by species. Trees native to Europe (e.g., *Quercus* spp.) have a richer lichen cover than those originated from other continents (e.g., *Sophora japonica* *Platanus hybrida*). Along busy streets or in small parks of built-up areas native trees are seldom planted.

Microclimate is of increased importance because in many cases lichens are found only at the foot of trees. Near the soil surface the air is wetter and the drying effects of the wind is less intensive than in the upper region.

Table 5

Number of taxa from different substrate types

| | Bark and wood (epiphytic) | Soil (epigeic) | Rock (epilithic) |
|-----------|---------------------------------|-------------------|---------------------|
| 1910–1915 | 64 | 56 | 120 |
| 1979–1982 | 81 | 44 | 55 |

The localization of the host trees within the city is important. In the hills the species number is higher than in the neighbouring flat areas. In Budapest lichens were found only in areas covered by natural vegetation or planted with native trees but not in the central built-up areas. Lichens are absent even from the parks that are usually not big enough to protect lichens from lead pollution. The extensive parks (e.g., Városliget, Népliget) are in zones highly polluted with sulphur dioxide.

There was no site with epilithic lichens present but epiphytic ones absent. The most frequently occurring epilithic species are *Squamaria albomarginata* (Nyl.) Räs., *Lecanora dispersa* (Pers.) Röhl. which were also found by Gallé in Szeged, and *Candelariella vitellina* (Ehrh.) Müll. Arg. while Gallé has found *C. aurella* (Hffm.) A. Z. (GALLÉ 1979).

Examining the number of taxa on different substrates at different times (Table 5), the data may appear contradictory. The decrease in the number of taxa on stones and soils is an existing trend. The increasing number of epiphytic species may be due to the fact that only the more colourful epilithic and epigeic taxa were collected at the beginning of the century, and the epiphytes were largely overlooked. In our study, however, mainly the less visible epiphytic lichens were examined.

Summary

The area of Budapest is mostly a lichen desert. As a transition toward the normal zone two struggle zones can be distinguished. The normal zone runs at best along the boundary of Budapest, but mostly beyond that.

The most resistant epiphytic lichen species found in both struggle zones are *Scoliciosporum chlorococcum*, *Lecanora conizaeoides*, *L. varia*, *Buellia punctata*, *Hypogymnia physodes* and *Parmelia sulcata*.

The inner struggle zone is characterized by the occurrence of *L. pini-perda* and *L. hageni* and the outer one by *L. sarcopis*, *L. subintricata* and *Lecidea lucida*.

Beyond the boundary of normal zone occurs *P. glabrata* var. *fuliginosa*.

Heavy metal (Pb, Cd, Mn, Zn) concentrations were measured in the epiphytic *H. physodes* and the epigeic *Cladonia convoluta* transplanted to 50 study sites within the city limits. The atomic absorption spectrophotometry carried out after exposure time of three months showed that lead content is usually higher near the roads than far apart.

Along some transects through the city lead content increases toward the centre.

In a short period of time *Cl. convoluta*, an epigeic fruticose lichen, accumulates more heavy metals than the corticolous *H. physodes*.

Extensive areas which were within the normal zone in 1910–15 have turned into a lichen desert by now.

Potential reasons for the impoverishment of lichen flora are the lead pollution caused by increased traffic, sulphur dioxide pollution originated from heating, the decrease of area covered by natural or seminatural vegetation, the increase of built-up areas, the warmer and drier urban climate and, finally, substrate effects.

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MONOSULCATE POLLEN GRAINS OF ANGIOSPERMS FROM HUNGARIAN ALBIAN SEDIMENTS, II

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While studying the Albian deposits of the Transdanubian Central Range, the authors found a number of new species of early monosulcate angiosperm pollen grains which they assigned, in 1984, to the new form genera described by them. In the present work they continue the description of these new species, assigning 7 new species to the genus *Crassipollis*, 2 ones each to *Transitoripollis* and *Oroszlanyipollis* and 1 to the genus *Similipollis*.

Keywords: Palynology, Middle Cretaceous, angiosperm pollen.

Introduction

During their study of early monosulcate pollen grains the authors first described species as nomina nuda (JUHÁSZ and GÓCZÁN 1976), but the description of new genera and species began only in their latest work (GÓCZÁN and JUHÁSZ 1984), when they described the monosulcate genera *Crassipollis*, *Transitoripollis* and *Similipollis* and the trichotomosulcate genus *Oroszlanyipollis* and identified 12 new species as representatives of these new genera. Started in the first paper, the description of species is continued in this one with presentation of the species left out of the first description and references are made to the valid genera and species already described.

SYSTEMATIC PART

Genus: *Crassipollis* Góczán et Juhász 1984

Crassipollis magnus nov. sp.

Plate I, Figs 1-12

Derivatio nominis: after its big size

Locus typicus: Pusztavám, borehole Ptv-2

Stratum typicum: 120.0-121.20 m, dark grey argillaceous marl, Tés Clay Formation, Middle Albian

Holotype: Slide 13948. Coord.: 9.5-123.0, Plate I, Figs 1-12.

Diagnosis: Pollen grain oval in E-view, of great size, oblate spheroidal, thick-exined, tectate-perforate, monosulcate with a longitudinal axis identical in size with, or just a little bit shorter than, the E-axis. Sulcus extending almost from wall to wall along P-axis. In the thin nexine there is a simple split growing wider along both ends. In the thick nexine, it forms a radial dissolving field accompanying the sulcus, being just a few microns wider than the

opening of the sulcus. Exine $5.0\ \mu\text{m}$ thick, tectate-perforate. It gets narrow along the sulcus. Structurally, it consists of a thicker, smooth nexine and a thinner, structured sexine. Nexine about $3\ \mu\text{m}$ thick, smooth, being finely granulate only in the dissolution field of the sulcus. Sexine 1.8 to $2.0\ \mu\text{m}$ thick, clavate, tectate-perforate. The columella and the tectum are of nearly the same thickness. The columella layer consists of dense, closely spaced, well-arranged rows of the bacula of the clavae, the tectum being composed of a perforated layer resulting from coalescence of the heads sitting at the tips of the bacula. The tubules representing the perforation attain scarcely 0.2 to $0.3\ \mu\text{m}$ in diameter.

Size range: length $44\ \mu\text{m}$, width $45\ \mu\text{m}$, wall thickness $5\ \mu\text{m}$, $P : E = 0.97 : 1$.

Differential diagnosis: *Crassipollis magnus* nov. sp. differs from the other *Crassipollis* form-species by its size and by the tectum-columella thickness ratios.

Occurrence: Tés Clay Formation (Middle Albian).

***Crassipollis noszkyi* nov. sp.**

Plate II, Figs 1–12 and 13–16

Derivatio nominis: dedicated to the memory of Dr. Jenő NOSZKY, former director of the Hungarian Geological Institute, distinguished researcher of Hungary's Lower Cretaceous deposits

Locus typicus: Pusztavám: borehole Ptv-2

Stratum typicum: 120.0 – 121.2 m, dark grey argillaceous marl, Tés Clay Formation, Middle Albian

Holotype: Slide 13948/2. Coord.: 5.0 – 115.5 , Plate II, Figs 1–12.

Diagnosis: Thick-exined, monosulcate pollen grain, elliptical in shape when viewed in the E-plane, of medium size, oblate and tectate-perforate, having a length of polar axis by 8 to $9\ \mu\text{m}$ shorter than the equatorial diameter.

The sulcus in the longitudinal axis extends from pole to pole. In the thin exine there is a simple split growing progressively wider towards one of the poles. Penetrating deep into the thick nexine, it forms a wide dissolving field there.

The exine is 4.5 to $5.5\ \mu\text{m}$ thick. The sulcus is flattened at both ends, being often a little bit thinner than along the E-axis.

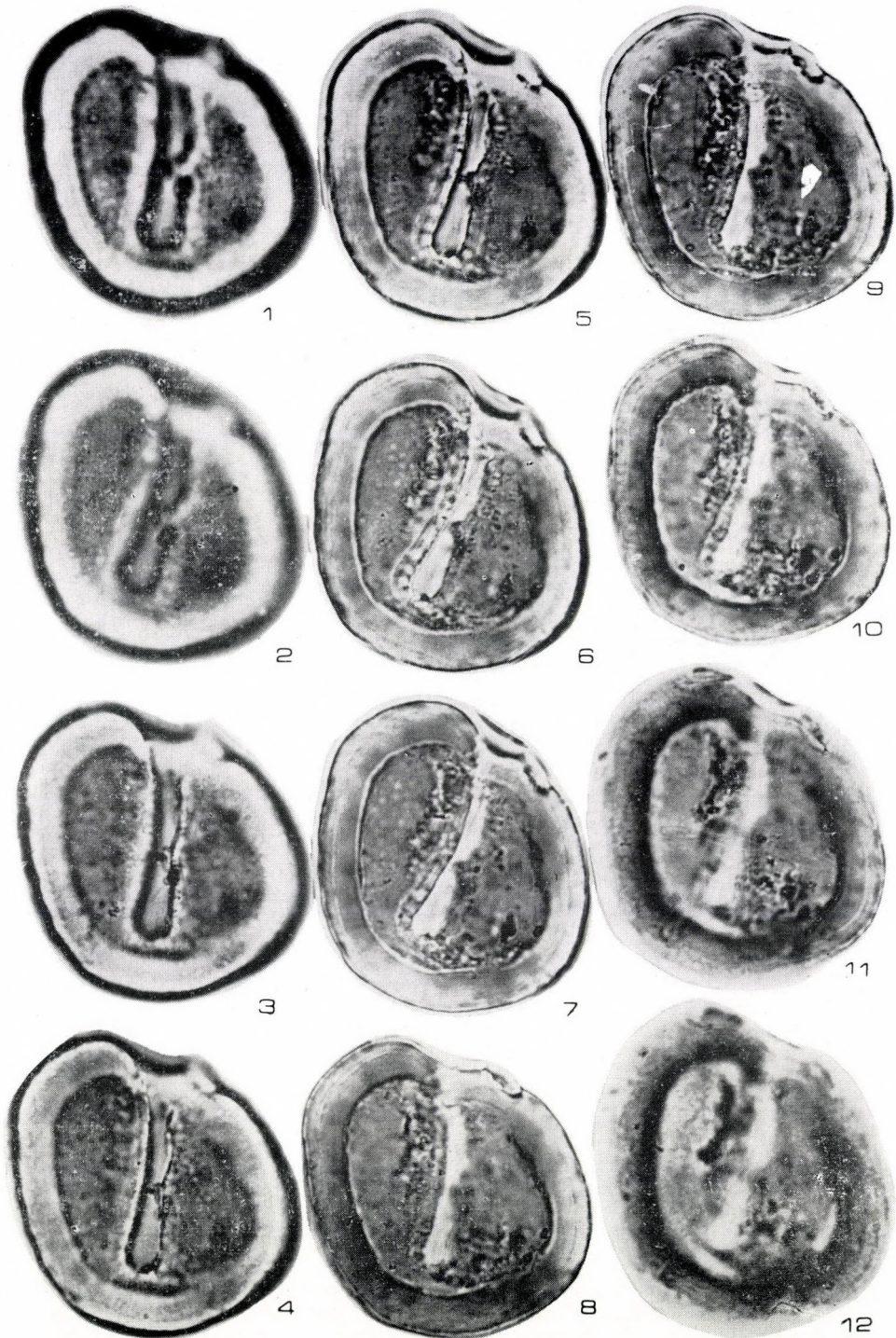
The nexine is 3 to 4 times as thick as the sexine. It is smooth, except for the dissolving field split in form of a wedge, often 3 – $5\ \mu\text{m}$ long, perpendicular to the sulcus, in the central area. Finely granulate in the dissolving field, its inner surface is covered by a 0.2 - to $0.3\text{-}\mu\text{m}$ -thick layer.

The sexine is thin, of clavate structure. The bacula forming the columella layer are about 0.6 to $0.7\ \mu\text{m}$ high, regularly arranged, and closely spaced. Resulting from coalescence of the heads of the bacula, the tectum is 0.3 to $0.4\ \mu\text{m}$ thick. The tubules piercing the sexine

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Plate I

Figs 1–12. *Crassipollis magnus* nov. sp. (holotype). Pusztavám, borehole Ptv-2, 120.0 – 121.20 m, *Munieria*-bearing argillaceous marl facies. Tés Clay Formation, Middle Albian. Grain in slide No. 13948, coord.: 9.5 – 123.0



have a lumen of 0.2 to 0.3 μm width and thus the sexine seems to be of negative reticulate structure.

Size range: largest diameter along the E-axis: between 27 and 29 μm , wall thickness between 4.5 and 6.0 μm .

Length of holotype: 21 μm , width of holotype: 29 μm , wall thickness along the poles: 3.5 μm , along the E-axis: 5.5 μm , P : E = 0.7 : 1.

Differential diagnosis: *Crassipollis noszkyi* n. sp. differs by its elliptical shape from both *Crassipollis pusztavamensis* Góczán et Juhász of irregularly square form and from the round *Crassipollis urkutensis* n. sp. From *Crassipollis deakae* Góczán et Juhász, a species of similarly, but more slightly, oval outline, it deviates with its smaller size and, at the same time, its perforation of larger lumen.

Occurrence: Tés Clay Formation (Middle Albian) and Pénzeskút Marl Formation (Upper Albian substuderer Zone).

***Crassipollis urkutensis* nov. f. sp.**

Plate II, Figs 17–24; Plate III, Figs 1–18

Derivatio nominis: after the type locality

Locus typicus: Urkút, borehole U-4

Stratum typicum: 44.4–45.0 m, grey marl, Pénzeskút Marl Formation, Upper Albian, Stoliczkaia dispar-Zone

Holotype: Slide I/2-2. Coord.: 8.2–102.8, Plate II, Figs 17–24.

Diagnosis: Thick-exined, monosulcate pollen grain, usually varying from the form of a slightly flattened circle up to an almost regular one, when viewed in the E-plane, of medium size, suboblate, tectate-perforate having a length of polar axis by 1 to 2 μm shorter than the equatorial diameter.

The sulcus extends from wall to wall along the longitudinal axis. In the thin sexine there is usually a narrow split which forms a dissolving field growing radially wider and having an inequally jagged edge in the thick nexine.

Exine 4.5 to 5.5 μm thick, tectate-perforate. Nexine about 3 to 4 μm thick, smooth over the larger part of the grain, being verrucate and granulate in the dissolving field along the sulcus. Its inner surface is covered by a smooth 1- μm -thick layer.

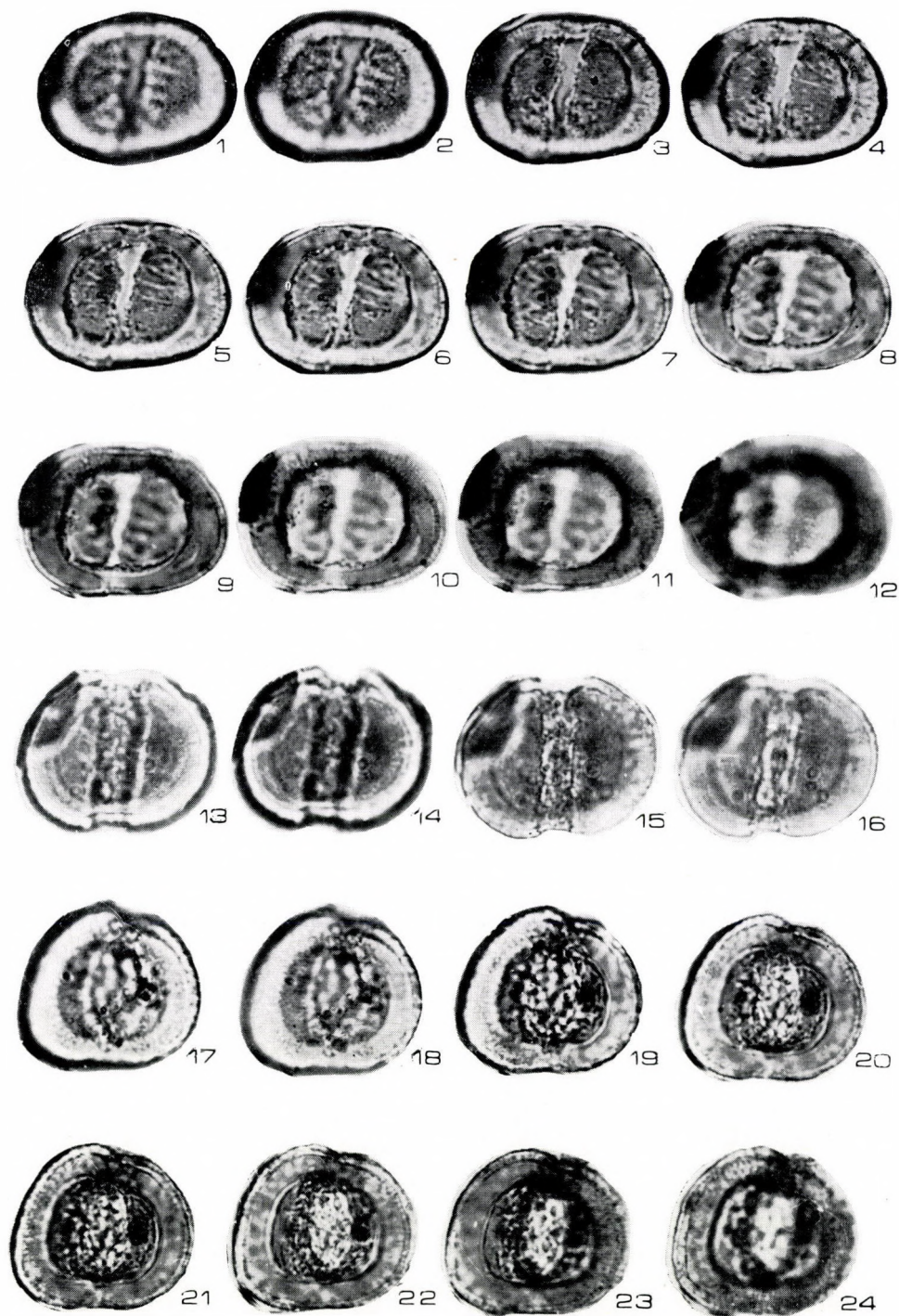
Sexine clavate, 1.0 to 1.5 μm thick. The columella is formed of a layer of the bacula of the clavae arranged by the 4 to 5 in a circular pattern tending, in the final analysis, to form dense, closely spaced rows.

Plate II

Figs 1–12. *Crassipollis noszkyi* nov. sp. (holotype). Pusztavám, borehole Pvt-2, 120.0–121.2 m. Tés Clay Formation, Middle Albian. Slide No. 13948/2, coord.: 5.0–115.5

Figs 13–16. *Crassipollis noszkyi* nov. sp. (paratype). Vértessomló, borehole Vst-5, 46.75–47.75 m, Vértessomló Siltstone Fm., mamillatum-Zone, Lower Albian. Slide No. 46.75/1, coord.: 35.8–105.1

Figs 17–24. *Crassipollis urkutensis* nov. sp. (holotype). Urkút, borehole U-4, 44.4–45.0 m, Pénzeskút Marl Fm., dispar-Zone, bergeri-Subzone, Upper Albian



The bacula are 0.6 to 0.8 μm high. The tectum is a perforate layer resulting from coalescence of the 0.4- to 0.7- μm -high heads of the clavae. The surface lumina of the tubules piercing the sexine are about 0.3 to 0.4 μm wide and, for this reason, the surface appears to be negatively microreticulate.

Size range: largest diameter 23 to 26 μm , wall thickness varying between 4 and 5.5 μm .

Size range of the holotype: along the P-axis: 24 μm , along the E-axis: 25 μm , height about 7–8 μm , wall thickness 5.5 μm , P : E = 0.9.

Differential diagnosis: *Crassipollis urkutensis* n. sp. differs by its more or less round shape, by its size range and the larger lumina of its perforations, from *Crassipollis deakae* Góczán et Juhász of larger size and more oval shape, its divergence from *Crassipollis pusztavamensis* Góczán et Juhász, species most closely related to it, consisting primarily in its coarser perforations and the outline in the E-plane. The E-contour of *Crassipollis pusztavamensis* Góczán et Juhász is a square with rounded angles and convex sides, that of *Crassipollis urkutensis* n. sp. being an almost regular circle.

Remark: On the SEMGs of the grain presented in Plate III, in spite of the worn surface, the perforations of the tectum are quite distinct and the size and spacing of the lumina are measurable.

Occurrence: From the upper part of the Tés Clay Formation up to the substuder- and dispar-Zones of the Pénteskút Marl Formation (Upper Albian).

***Crassipollis longisulcatus* nov. sp.**

Plate IV, Figs 1–6

Syn.: *Crassipollis longisulcatus* Juhász et Góczán 1976, p. 38. Pl. 1 : 9 (nomen nudum)

Derivatio nominis: after its sulcus

Locus typicus: Pusztavám, borehole Pvt-2

Stratum typicum: 120.0–121.2 m, dark grey argillaceous marl, Tés Clay Formation, Middle Albian

Holotype: Slide 13948/1. **Coord.:** 9.1–122.0, Plate IV, Figs 1–6

Diagnosis: Thick-exined, subprolate, tectate-perforate, monosulcate pollen grain, oval when viewed in the E-plane, having a polar axis by 4 μm longer than the equatorial diameter.

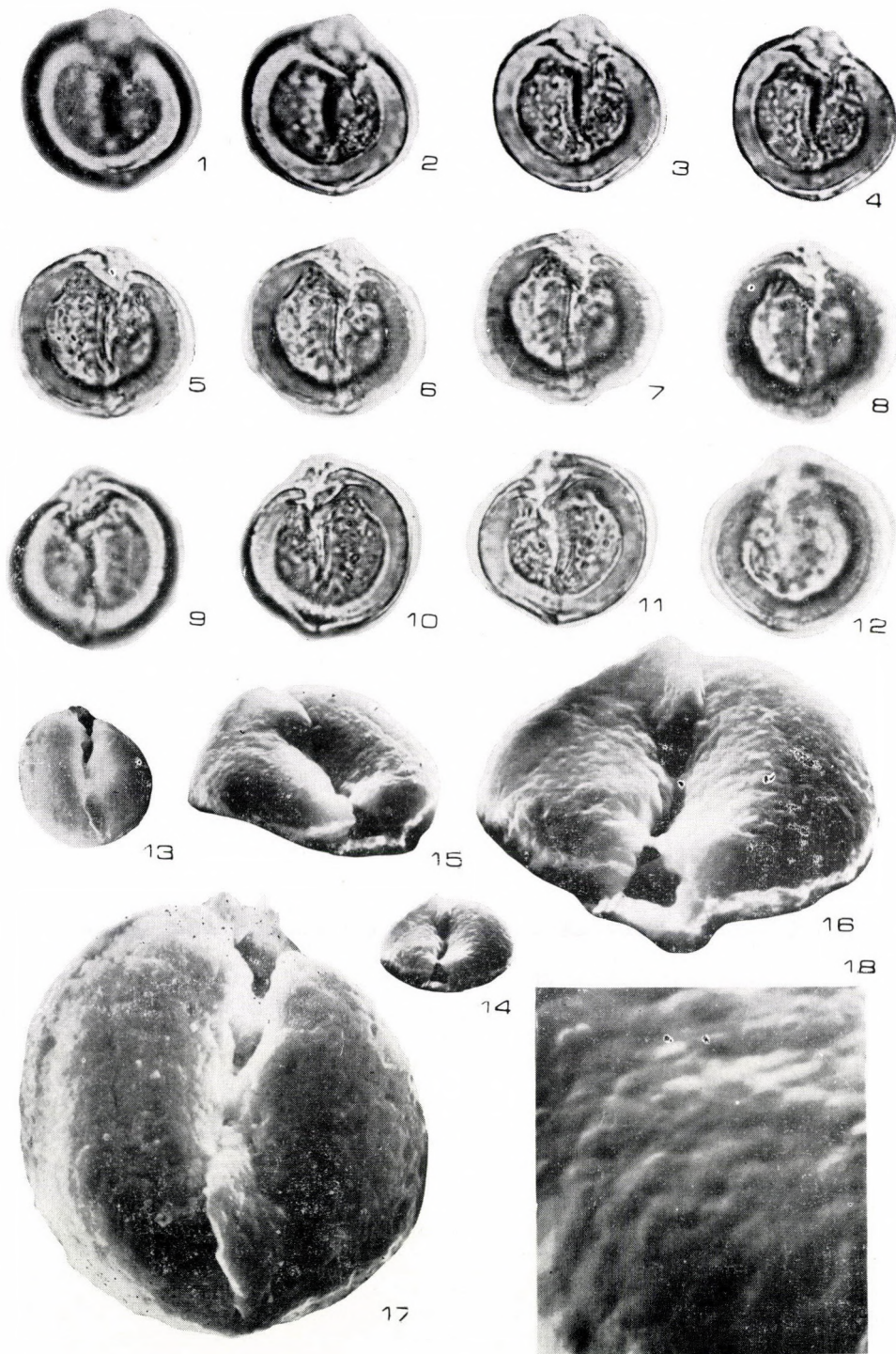
The sulcus extends from wall to wall along the polar axis, forming a simple, narrow split in the very thin sexine and an about 5- to 6- μm -wide, slightly jagged dissolving field in the nexine.

Plate III

Figs 1–8. *Crassipollis urkutensis* nov. sp. (paratype). In glycerine droplet slide, photographed from one side. Urkút, borehole U-5, 71.2–73.2 m, Munieria-bearing argillaceous marl facies, Tés Clay Fm., Middle Albian

Figs 9–12. The same grain, photographed from the other side

Figs 13–18. The same grain, SEMGs: 13, 17, in nearly the E-plane (13 = $\times 1000$, 17 = $\times 3000$) 14, 16 = from the cracked aperture of the sulcus (14 = $\times 1000$, 16 = $\times 3000$) — 15 = half-side view ($\times 1800$); 17 = E-plane ($\times 3000$) — 18 = detail of surface of proximal side, surface of grain strongly affected by tear and wear in the E-plane ($\times 10\,000$)



Exine 2.0 to 2.2 μm thick, being a little bit thicker along the P-axis than in the E-axis, slightly compressed at one of its ends. Nexine 1.6 to 1.7 μm thick, smooth, structureless, only the inner cover-lamella of about 0.3 μm thickness seems to be sculptured and, in the dissolving field of the sulcus, punctate.

The sexine is 0.4 to 0.5 μm thick, the thickness of the tectum and the columella being immeasurable by the light microscope. The tubulate perforations visible on its surface have a lumen of about 0.1 μm size.

Size range: length: 21 μm , width: 16 μm , wall thickness: 2.0–2.2 μm . P : E index: 1.23, P : e = 10.5 (e = exine thickness).

Differential diagnosis: With its different shape, size range and sexine structure, *Crassipollis longisulcatus* nov. sp. differs quite distinctly from the other similar *Crassipollis* species. From *Crassipollis pyriformis* nov. sp., a similarly subprolate form standing closest to it, *Crassipollis longisulcatus* nov. sp. is made distinct primarily by the difference in the perforation of the sexine. The perforations of *Crassipollis pyriformis* n. sp. have lumina of 0.7 to 0.8 μm size, those of *Crassipollis longisulcatus* n. sp. being below 0.1 μm .

Occurrence: Tés Clay Formation (Middle Albian).

***Crassipollis minimus* nov. sp.**

Plate IV, Figs 7–19

Syn.: *Crassipollis minimus* Juhász et Góczán 1976, p. 38, Pl. 1 : 7 (nomen nudum)

Derivatio nominis: after its size

Locus typicus: Oroszlány, borehole O-1825

Stratum typicum: 211.0 m, gray glauconitic marl, Pénzeskút Marl Formation, Upper Albian, Lower Vraconian substuderi-Zone

Holotype: Slide 17344/1. Coord.: 29.4–108.8, Plate IV, Figs 7–19.

Diagnosis: Thick-exined, tectate-perforate, monosulcate pollen grain of small size, round or slightly oval, oblate-spheroidal when viewed in the E-plane, having a polar axis by 1–2 μm shorter than the equatorial diameter.

The sulcus is straight, extending from pole to pole, with a 2 μm wide split in the sexine, growing all of a sudden wide at one of the poles. In the thick nexine it forms a slightly disintegrated, though sculptured, dissolving field.

Plate IV

Figs 1–6. *Crassipollis longisulcatus* nov. sp. (holotype). Pusztavám, borehole Pvt-2, 120.0–121.2 m, Tés Clay Fm., Middle Albian. Slide No. 13948/1, coord.: 9.8–122.0

Figs 7–19. *Crassipollis minimus* nov. sp. (holotype). Oroszlány, borehole O-1825, 211.0 m. Pénzeskút Marl Fm., blancheti-Subzone, Upper Albian. Slide 211/3, coord.: 8.2–102.9

Figs 20–21. *Crassipollis tesensis* nov. sp. (paratype). Pénzesgyőr, borehole Pgy-4, 69.7 m. Pénzeskút Marl Fm., substuderi-Zone, Upper Albian. Slide No. 69.7/9, coord.: 38.8–102.2

Figs 22–31. *Crassipollis tesensis* nov. sp. (holotype). Tés, borehole Tt-27, 50.5 m. Tés Clay Fm., Middle Albian. Slide No. T/1–2, coord.: 20.8–105.2

Figs 32–36. *Crassipollis pyriformis* nov. sp. (holotype). Pusztavám, borehole Pvt-2, 121.20–124.7 m. Tés Clay Fm., Middle Albian. Slide 13952/3, coord.: 10.0–116.4



The exine is about $3\text{ }\mu\text{m}$ wide, narrowing slightly along the sulcus. The nexine is 2.6 to $2.7\text{ }\mu\text{m}$ wide, smooth, structureless, only along the sulcus are there a few narrow, radially oriented cracks. The inner surface is covered by a smooth lamella, 0.3 to $0.4\text{ }\mu\text{m}$ thick. The sexine is 0.3 to $0.4\text{ }\mu\text{m}$ thick, the thickness of the columella and the tectum being immeasurable, though of seemingly identical size. The perforation of the tectum is at the limit of the resolution power of the light microscope, having a lumen below about $0.1\text{ }\mu\text{m}$.

Size range: length $17\text{ }\mu\text{m}$, width $19\text{ }\mu\text{m}$, wall thickness $3\text{ }\mu\text{m}$, P : E index: 0.89, P : e index = 5.6.

Differential diagnosis: With its size, shape and proportions and the pattern of the sulcus, *Crassipollis minimus* nov. sp. differs quite distinctly from any *Crassipollis* species of small size.

Remark: In terms of its size, shape and exine structure, *Crassipollis minimus* nov. sp. seems to be a transition between the genera *Transitoripollis* and *Crassipollis*. On the basis of its P : e index and the pattern of the sulcus, however, the author judges it to belong to the genus *Crassipollis*.

Occurrence: Lower part of the Pénzeskút Marl Formation (Lower Vraconian, Upper Albian).

***Crassipollis tesensis* nov. sp.**

Plate IV, Figs 20–31

Syn.: *Transitoripollis vulgaris* Juhász et Góczán 1976, p. 38, Pl. 1 : 6 (nomen nudum)

Derivatio nominis: after its type locality

Locus typicus: Tés, borehole T-27

Stratum typicum: 50.0 m, dark grey argillaceous marl, Tés Clay Formation, Middle Albian

Holotype: Slide I/1–2. Coord.: 20.8–105.2, Plate IV, Figs 22–31.

Diagnosis: Thick-exined, tectate-perforate, monosulcate pollen grain, elliptical in shape, prolate-spheroidal when viewed along the E-axis.

Its sulcus extends from wall to wall along the P-axis. In the thin sexine there is a simple split which grows wider in its median line. In the thick nexine it forms a radially wide, oval, punctate-granulate dissolving field.

Exine 2.0 to $2.2\text{ }\mu\text{m}$ thick. It consists of an unsculptured, thick inner layer and a sculptured and thin outer one. The shape at the ends of the sulcus grows gradually convex rather than concave. The nexine is about $1.8\text{ }\mu\text{m}$ thick, being punctate-granulate in the dissolving field along the sulcus and smooth over the larger part of the surface. Its inner surface is covered by a smooth lamella 0.2 to $0.3\text{ }\mu\text{m}$ thick. The sexine is 0.4 to $0.5\text{ }\mu\text{m}$ thick, seemingly tectate-perforate (foveolate?), being impossible to determine for sure with the light microscope. The lumina of perforations (?) are around 0.1 – $0.2\text{ }\mu\text{m}$ in diameter.

Size range: length $22\text{ }\mu\text{m}$, width $20\text{ }\mu\text{m}$, wall thickness $2.2\text{ }\mu\text{m}$, P : E index = 1.1, P : e index = 9.1

Differential diagnosis: *Crassipollis tesensis* nov. sp. resembles to *Crassipollis dissimilis* Góczán et Juhász, *Crassipollis pyriformis* n. sp. and *Crassipollis minor* Góczán et Juhász, by its shape, and size range. However, it differs

from all three by the fact that the exine is not compressed at any of the ends of the polar axis and that neither end of the sulcus is widened. The circumstance that the sulcus is widened at its median rather than at the poles shows its being akin to some representatives of the genus *Transitoripollis*; however, its punctate-granulate dissolving field in the exine makes it quite distinct even from these.

Remark: Because of our incertitude about the tectate-perforate structure of the sexine of *Crassipollis tesensis* nov. sp. and of the deviation of the character of its sulcus in this layer from the other *Crassipollis* nov. sp., we have only provisionally assigned it to this genus. That it may derive from Cycadinae rather than Angiosperma is not implausible either.

Occurrence: Tés Clay Formation (Middle Albian).

***Crassipollis pyriformis* nov. sp.**

Plate IV, Figs 32–36

Syn.: *Crassipollis pyriformis* Juhász et Góczán 1976, p. 38, Pl. 1 : 10 (nomen nudum)

Derivatio nominis: after its shape

Locus typicus: Pusztavám, borehole Ptv-2

Stratum typicum: 121.2–124.7 m, dark grey argillaceous marl, Tés Clay Formation, Middle Albian

Holotype: Slide 13952/3. Coord.: 10.0–116.4, Plate IV, Figs 32–36.

Diagnosis: Thick-exined, subprolate, tectate-perforate, monosulcate pollen grain of medium size, ellipsoidal, strongly flattened at one of its poles, when viewed in the E-plane, along the longitudinal axis.

The sulcus extends from wall to wall along the P-axis. In the thin sexine there is a simple, narrow split; in the thick nexine, it forms, radially, on both sides of the aperture and along it, a 3 to 4 μm wide dissolving field.

The exine is about 4 μm thick, becoming thinner along the sulcus and at its tips. It consists quite distinctly of a thicker, smooth inner layer and a thinner and structured outer one.

The nexine is about 3.4 μm thick. It is smooth and structureless, except for the dissolving field along the sulcus, where it appears to be verrucate. Its inner surface is covered quite distinctly by an about 0.5- μm -thick, and smooth inner layer.

The sexine is about 0.6 μm thick and clavate. The bacula forming the columella layer are arranged into short, but widely spaced and quite distinct rows. Formed as a result of coalescence of the heads sitting at their tips, the perforate tectum seems to be microreticulate. The columella and the tectum appear to be of nearly identical height. The lumen of tubules at their reaching the surface has a diameter of 0.7 and 0.8 μm , being larger than the interval in-between.

Size range: length 24 μm , width 20 μm , wall thickness 4 μm , P : E = 1.2.

Differential diagnosis: The difference of *Crassipollis pyriformis* n. sp. from the other *Crassipollis* f. sp. consists in its peculiar elliptical shape and the relatively large lumina of the perforations. This holds true of the case of

Crassipollis longisulcatus n. sp., a form standing very close to the former and similarly subprolate in which the lumina of the tubules are below $0.1\ \mu\text{m}$ so that the tectum appears to be imperforate. The difference from *Crassipollis dissimilis* Góczán et Juhász, another similar form, lies in its more slender shape and its perforation having an about twice so large diameter of lumen.

Remark: As observable quite clearly on the type specimen, the perforation is arranged into rows not only along the E-axis, but parallel to the sulcus as well.

Occurrence: Tés Clay Formation (Middle Albian).

***Crassipollis chaloneri* (Brenner 1963) nov. comb.**

Plate VI, Figs 35–39

Type and diagnosis: Brenner 1963, p. 73, Pl. 24, Figs 9–11.

Remark: *Monosulcites chaloneri* Brenner is similar to the *Crassipollis* species described by the present writer as far as its structure of the sulcus, its size proportions and its wall structure are concerned.

Genus: *Transitoripollis* Góczán et Juhász 1984

***Transitoripollis vulgaris* nov. sp.**

Plate V, Figs 1–19; Plate VI, Figs 1–10

Locus typicus: Pusztavám, borehole Pvt-2

Stratum typicum: 120.0–121.2 m, grey argillaceous marl, Tés Clay Formation, Middle Albian

Holotype: Slide 13948/1. Coord.: 9.1–109.8, Plate V, Figs 1–19.

Diagnosis: Thin-exined, intectate by light microscope, monosulcate pollen grain of small size, oblate-spheroidal, less frequently subprolate or prolate, round or slightly oval in outline, when viewed in the E-plane.

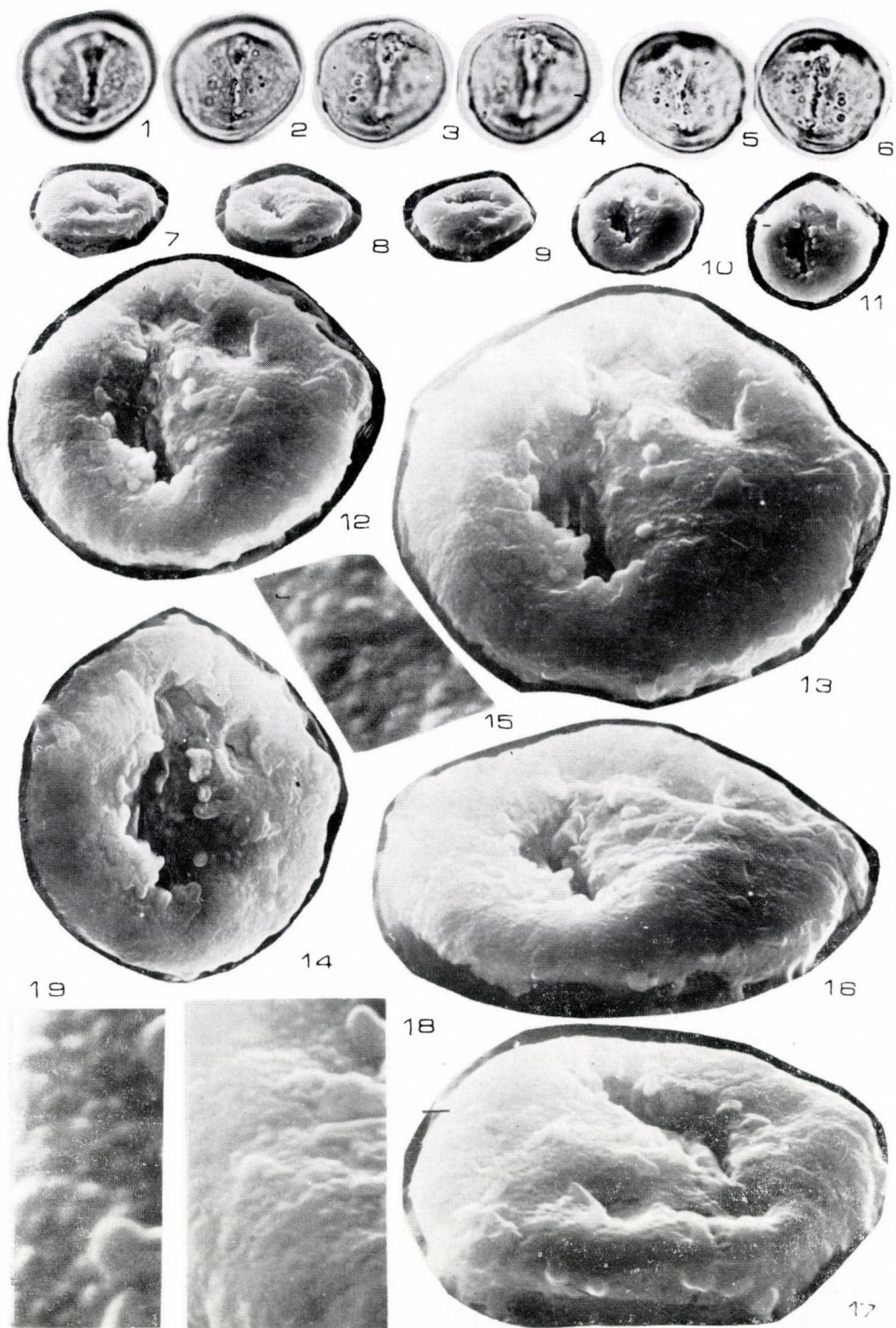
Its sulcus extends along the longitudinal axis, representing a simple split usually a few microns shorter than that, being often widely open, in the same way, both in the sexine and the nexine.

Plate V

Transitoripollis vulgaris nov. sp. (holotype)

Figs 1–6. Single grain preparation, light micrographs, photographed with an objective of 100/1.40 ($\times 1000$). Pusztavám, borehole Pvt-2, 120.0–121.20 m, Munieria-bearing grey argillaceous marl facies, Tés Clay Fm., Middle Albian. 1–4 = between two cover-plates, as photographed from the one side, 5–6 = as photographed from the other side

Figs 7–19. The same grain, SEMgs (7–11 = $\times 1000$; 12, 14 = $\times 3000$; 13, 16, 17 = $\times 4000$; 18 = $\times 10\ 000$; 15, 19 = $\times 20\ 000$). On the micrographs of a magnification of $\times 10\ 000$ and $\times 20\ 000$ the microverrucate ultrasculpture is quite distinct. The verrucae are arranged in a circular pattern by the four and five, similarly to the case of *Crassipollis* and, arranged in rows on the surface, they extend towards the sulcus. In Figs 12 and 13 one can see on the surface the sulcus penetrating deep into the nexine and piercing it as well as a few coarse alien grains



The exine is 1.0 to 1.5 μm thick, consists of two more or less equally thick layers.

The nexine is 0.5 to 0.8 μm thick, smooth, unsculptured, having no dissolving field along the sulcus.

The sexine is more or less equal in thickness to the nexine. Intectate, its surface seems to be chagrenate when viewed by the aid of light microscope. When examined by SEM techniques, its ultrasculpture is microverrucate. The microverrucae are about 0.1 μm in diameter, being solitary or intergrown in twos along the base. They cover the entire surface uniformly. Their arrangement resembles to the case of *Crassipollis*.

Size range: length 18–21 μm , width 16.5–20 μm , wall thickness 1.0–1.5 μm .

The holotype is 18–19 μm long, 19 μm wide, its wall thickness being about 1.2 μm , $P : E = 0.95$, $P : e = 15$.

Differential diagnosis: It is in the first place its greater size and lower index-numbers that distinguish *Transitoripollis vulgaris* nov. sp. from *T. similis* Góczán et Juhász, species showing the greatest resemblance to it. *T. vulgaris* has an average size of 19 μm (as measured for 10 grains), its $P : e$ index being 15, while in the case of *T. similis*, the same figure is 16.5 μm and 16, respectively. It is quite possible that there will be no need in the future to differentiate the two species, inasmuch as further research may lead consistently to the discovery of transitional specimens. Until this is achieved, we propose, however, to retain the two categories.

Occurrence: Tés Clay Formation to Pénzeskút Marl Formation, dispar-Zone (Middle and Upper Albian).

***Transitoripollis ovalis* nov. f. sp.**

Plate VI, Figs 11–34

Derivatio nominis: after its E-contour

Locus typicus: Oroszlány, borehole O-1891

Stratum typicum: 529.1–532.0 m, dark grey argillaceous marl, Tés Clay Formation, Middle Albian

Plate VI

Figs 1–5. *Transitoripollis vulgaris* nov. sp. (paratype). Pusztavám, borehole Pvt-2, 120.0–121.20 m. Tés Clay Fm., Middle Albian. Slide No. 13948/1, coord.: 19.7–112.5

Figs 6–10. *Transitoripollis vulgaris* nov. sp. (paratype). Pusztavám, borehole Pvt-2, 120.0–121.2 m, Tés Clay Fm., Middle Albian. Slide No. 13948/1, coord.: 6.6–115.0

Figs 11–16. *Transitoripollis ovalis* nov. sp. (holotype). Oroszlány, borehole O-1891, 529.1–532.0 m, Tés Clay Fm., Middle Albian. Slide No. 529.1/2, coord.: 24.0–115.6

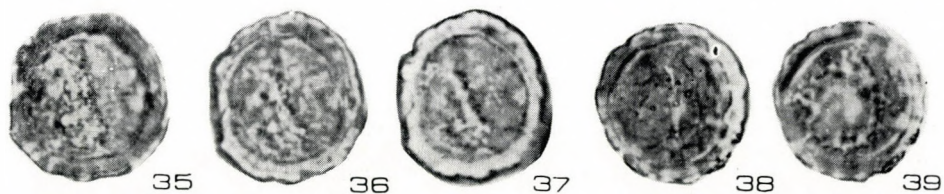
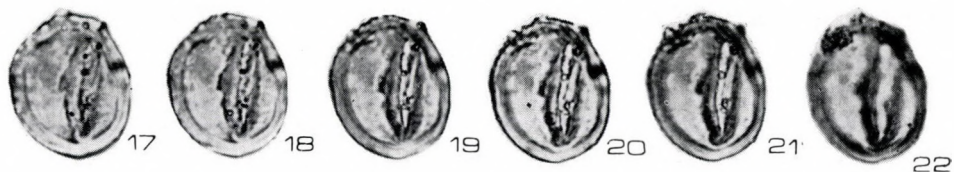
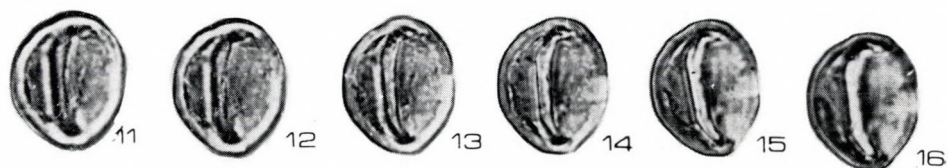
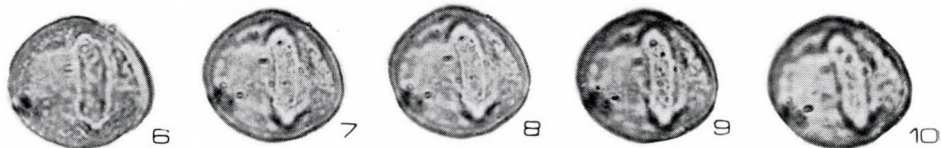
Figs 17–22. *Transitoripollis ovalis* nov. sp. (paratype). Pusztavám, borehole Pvt-2, 120.0–121.2 m, Tés Clay Fm., Slide No. 13948/1, coord.: 21.9–107.8

Figs 23–27. *Transitoripollis ovalis* nov. sp. (paratype). Tés, borehole Tt-27, 50.5 m. Tés Clay Fm., Middle Albian, Slide No. T/5-3, coord.: 2.8–96.0

Figs 28–34. *Transitoripollis ovalis* nov. sp. (paratype). Pusztavám, borehole Pvt-2, 120.0–121.2 m, Tés Clay Fm., Middle Albian

Figs 35–37. *Crassipollis chaloneri* (Brenner 1963) nov. comb. Oroszlány, borehole O-1825, 213 m, Pénzeskút Marl Fm., blancheti-Subzone, Upper Albian. Slide No. 213/2; coord.: 9.8–11.0

Figs 38–39. *Crassipollis chaloneri* (Brenner 1963) nov. comb. Pusztavám, borehole Pvt-2, 120–121.2 m. Tés Clay Fm., Middle Albian, Slide No. 13948/1, coord.: 15.0–102.0



Holotype: Slide 529/3. Coord.: 24.0–115.6, Plate VI, Figs 11–16.

Diagnosis: Thin-exined, intectate by light microscope, monosulcate pollen grain of small size, prolate and oval in outline, when viewed in the E-plane. Its sulcus extends along the longitudinal axis, usually from wall to wall, representing a simple split, piercing both layers of the exine in the same way and forming no dissolution field in the nexine.

The exine is 1.2 to 1.6 μm thick, consisting of two more or less equally thick layers adhering closely to each other. The nexine is about 0.6 to 0.8 μm in thickness, smooth, structureless, bearing no dissolving field along the sulcus. The sexine is of more or less equal thickness as the nexine. When viewed by the aid of a light microscope, its surface appears to be chagrenate. When examined by SEM techniques, it is tectate and microverrucate in terms of ultrasculpture.

The verrucae are about 0.1 μm in size, being intergrown, usually in twos or threes, along their base, standing sometimes alone, densely juxtaposed. They cover the entire surface uniformly.

Size range: length 16–21 μm , width 13–19 μm , wall thickness 1.2–1.6 μm .

Size range of the holotype: length 18 μm , width 15 μm , wall thickness 1.2, $P : E = 1.2$, $P : e = 15$.

Differential diagnosis: With its oval shape and characteristic sulcus and $P : E$ index, *Transitoripollis ovalis* nov. sp. readily deviates from the other *Transitoripollis* species of similar size. So does it, with its E-contour and its sulcus, from *Transitoripollis similis* and, with its sulcus and $P : E$ index, from *Transitoripollis anulisulcatus* Góczán et Juhász, another similar species.

Occurrence: Tés Clay Formation (Middle Albian).

Genus: *Oroszlanyipollis* Góczán et Juhász 1984

***Oroszlanyipollis baconicus* n. sp.**

Plate VII, Figs 1–13

Derivatio nominis: after the main facies area of the stratum typicum

Locus typicus: Urkút, borehole U-5

Stratum typicum: 71.2–73.2 m, grey argillaceous marl, Tés Clay Formation, Middle Albian

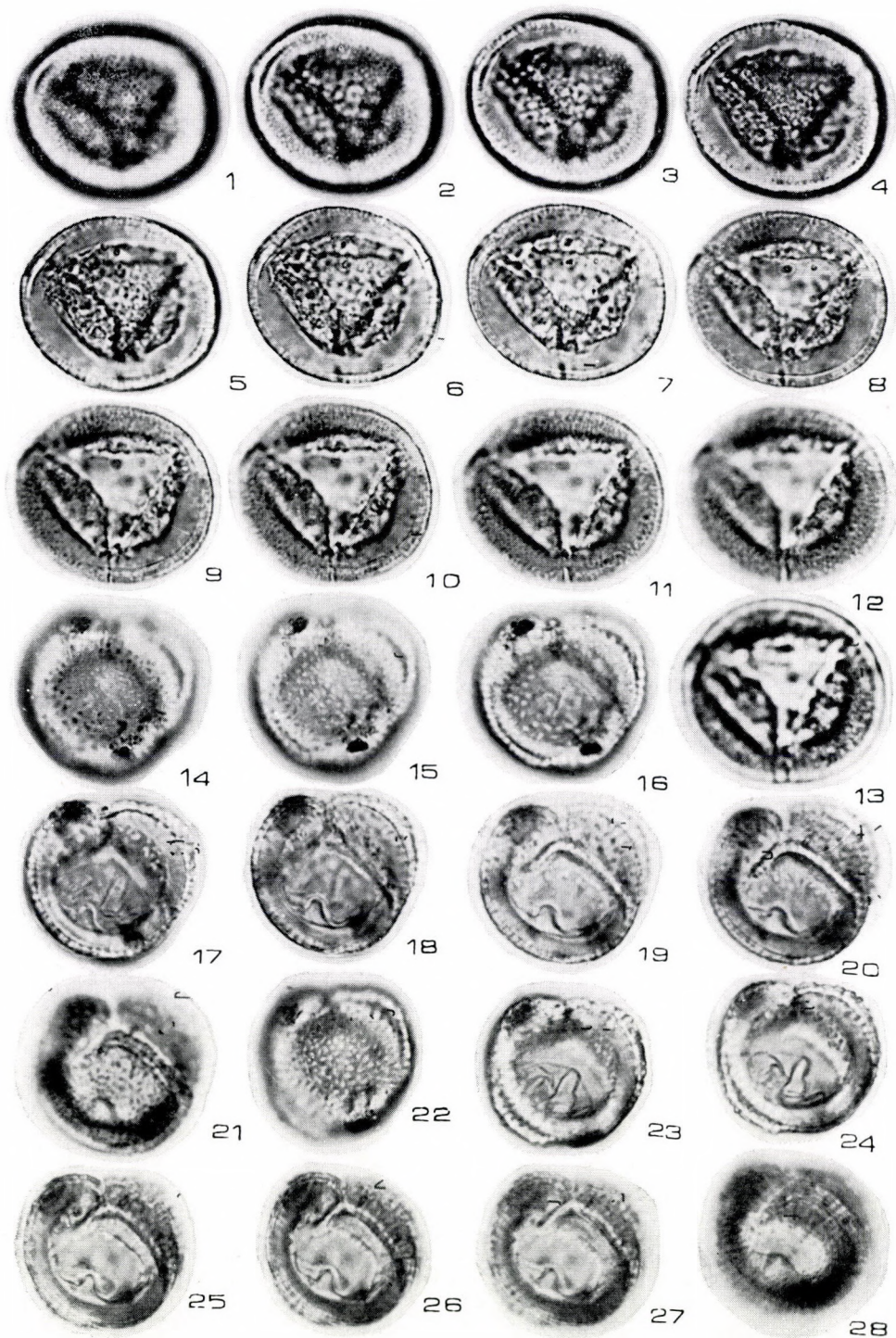
Holotype: Slide 71.2/5. Coord.: 15.3–114.2, Plate VII, Figs 1–13.

Diagnosis: Thick-exined, tectate, microreticulate, trichotomosulcate pollen grain of medium size, suboblate, triangular in shape with rounded angles and convex sides when viewed in the E-plane. Its Y-shaped sulcus between the poles extending from wall to wall, being open, piercing, in the same way, both the sexine and the nexine. Its exine is 5 μm thick,

Plate VII

Figs 1–13. Oroszlanyipollis baconicus nov. sp. (holotype). Urkút, borehole U-5, 71.2–73.2 m, Muniéria-bearing grey argillaceous marl facies; Tés Clay Fm., Middle Albian. Slide No. 71.2/5, coord.: 15.3–114.2

Figs 14–28. Oroszlanyipollis saparensis nov. sp. (holotype). Borehole Szápár-42, 119.1–121.1 m, Turritilites-bearing grey marl facies; Pénteskút Siltstone Fm., Lower Cenomanian, mantelli-Zone. Slide No. 8028/1, coord.: 20.4–124.2



getting thin at the poles, at the ends of the sulcus and consisting of a thick, smooth inner layer and a thin, structured outer one. Its nexine is $3.5\text{ }\mu\text{m}$ thick, smooth, compact and structureless; only along the lumen of the sulcus does it bear a microverrucate sculpture of secondary origin.

The sexine is about $1.5\text{ }\mu\text{m}$ thick, clavate and tectate-microreticulate.

The columella layer is composed of about $1\text{ }\mu\text{m}$ high bacula of the clavae, the tectum consisting of $0.5\text{-}\mu\text{m}$ -high heads of the clavae and of the muri connecting them. The reticulum is identical with the diameter of the lumen, covering uniformly and evenly the whole grain surface.

Size range: length $25\text{ }\mu\text{m}$, width $28\text{ }\mu\text{m}$, wall thickness $5\text{ }\mu\text{m}$, lumen diameter about 0.3 to $0.5\text{ }\mu\text{m}$, $P : E = 0.89$, $P : e = 5$.

Differential diagnosis: It is by its size, shape and the makeup of the reticulum that *Oroszlanyipollis baconicus* differs from *O. grandis* Góczán et Juhász, a species of much greater size and reticulum. Its difference from *O. saparensis* n. sp. of similar size lies, in turn, in addition to its shape, in its thicker exine and its finer reticulum of smaller diameter of lumen.

Occurrence: Tés Clay Formation (Middle Albian).

***Oroszlanyipollis saparensis* nov. sp.**

Plate VII, Figs 14–28

Derivatio nominis: after its type locality

Locus typicus: Szápár, borehole Sz-42

Stratum typicum: 119–121 m, grey marl, Pénezskút Siltstone Formation, Lower Cenomanian, mantelli–Zone

Holotype: Slide 8028/1. Coord.: 20.4–124.2, Plate VII, Figs 14–28.

Diagnosis: Thick-exined, tectate-microreticulate, trichotomosulcate pollen grain of medium size, rounded to oblate-spheroidal in outline when viewed in the E-plane.

Its sulcus extends from wall to wall, opening in the form of a narrow split in both layers of the exine.

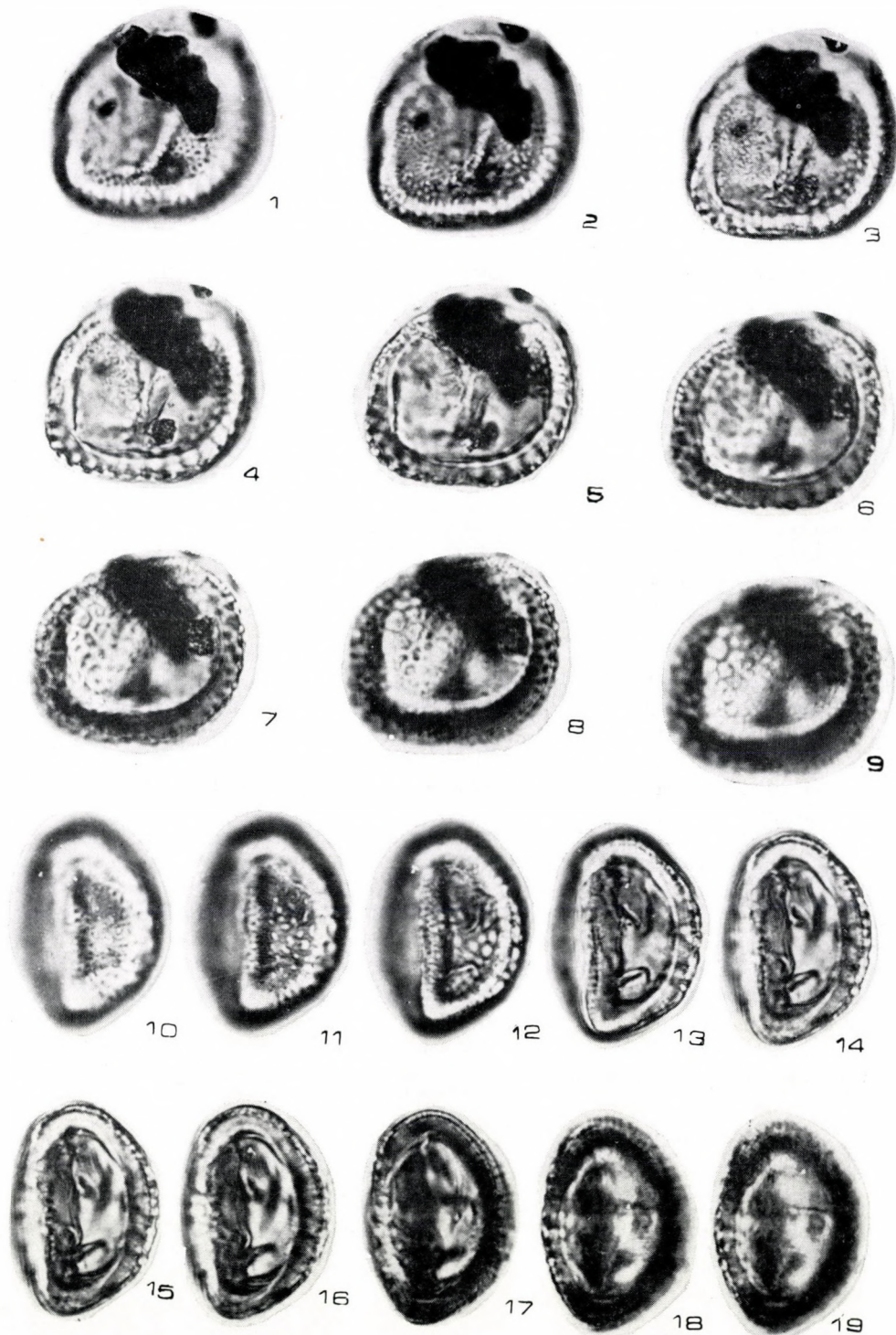
Its exine is $4\text{ }\mu\text{m}$ thick, consisting of a thinner, structured outer layer and a thicker, structureless, inner one. The adhesion of the two layers to each other is less tight. The nexine is about 2.7 to $2.8\text{ }\mu\text{m}$ thick, smooth, structureless, becoming thin along the sulcus, but having no dissolving field. The sexine is 1.2 to $1.3\text{ }\mu\text{m}$ thick, clavate, tectate-reticulate.

The columella consists of 1 to $1.1\text{ }\mu\text{m}$ high and 0.2 to $0.3\text{ }\mu\text{m}$ thick bacula, the tectum being composed of verrucae of 0.2 to $0.3\text{ }\mu\text{m}$ size forming the heads of the bacula and, respectively, of a web of muri, 0.3 to $0.5\text{ }\mu\text{m}$ thick, connecting these in a network pattern.

Plate VIII

Figs 1–9. *Similipollis orbiculatus* nov. sp. (holotype). Bakonyháza, surface exposure, Sample II/1–2; Pénezskút Siltstone Fm., Upper Vraconian, dispar–Zone, bergeri–Subzone. Slide No. II61–3, coord.: 15.1–11.3, approximately in the E-plane

Figs 10–19. *Similipollis orbiculatus* nov. sp. (paratype). Hárskút, borehole Hk-4, 127.9–130.6 m, Turritiles-bearing marl facies, Pénezskút Siltstone Fm., Upper Vraconian, dispar–Zone, bergeri–Subzone. Slide No. 45/3, coord.: 16.0–115.4, side view



The diameter of lumen of the reticulum is the same throughout the surface, being around $0.5\ \mu\text{m}$.

Size range: length $23\ \mu\text{m}$, width $24\ \mu\text{m}$, wall thickness $4\ \mu\text{m}$, $P : E = 0.96$, $P : e = 5.7$.

Differential diagnosis: It is by its much smaller size, by its proportions and shape that *Oroszlanyipollis saparensis* n. sp. differs from *O. grandis*. With its shape and its thinner wall and coarser reticulum, it deviates, in turn, from *O. baconicus* n. sp. as well.

Occurrence: Pénezskút Siltstone Formation, mantelli—Zone (Lower Cenomanian).

Genus: *Similipollis* Góczán et Juhász 1984

***Similipollis orbiculatus* nov. f. sp.**

Plate VIII, Figs 1–19

Derivatio nominis: after its round E-contour (orbiculatus = round in Latin)

Locus typicus: Hárskút, borehole Hk-4

Stratum typicum: 44.4–45.0 m, grey marl, Pénezskút Siltstone Formation, Upper Vraconian, dispar—Zone, bergeri-subzone

Holotype: Slide 467/3. Coord.: 43.5–102.0, Plate VIII, Figs 1–19.

Diagnosis: Thick-exined, tectate-reticulate, monosulcate pollen grain of medium size, round or slightly oval in outline in the E-plane, suboblate and oblate-spheroidal.

Its sulcus extends from wall to wall, mostly tapering at its ends, representing a simple split slightly widening along the median line and piercing, in the same way, both exine layers and forming no sculptured dissolving field in the nexine.

The exine is $4\text{-}\mu\text{m}$ thick, consisting of a smooth, unsculptured, thicker inner layer and a sculptured and thinner outer one.

The nexine is $3\ \mu\text{m}$ thick, smooth, compact, unsculptured, the sexine being about $1\ \mu\text{m}$ thick, tectate-reticulate. The columella consist of squat, 0.5- to $0.6\text{-}\mu\text{m}$ -high and thick bacula emerging from the nexine, the tectum being composed of a web of muri connecting the tips of the bacula. These are shorter and more closely spaced on the one side than on the other.

The diameter of lumen of the reticulum is on the side having a sulcus smaller than it is on the opposite side.

Size range: length 28 to $32\ \mu\text{m}$, width 28 to $32\ \mu\text{m}$, wall thickness $4\ \mu\text{m}$, $P : E = 1\text{--}1.1$, $P : e = 7\text{--}8$. Length of holotype: $30\ \mu\text{m}$, its width $30\ \mu\text{m}$, wall thickness $4\ \mu\text{m}$, $P : E = 1$, $P : e = 7.5$, sexine : nexine = 1 : 3.

Differential diagnosis: It is by its smaller size and more squat reticulum and its sexine : nexine ratio that *Similipollis orbiculatus* nov. fsp. differs from *Similipollis varireticulatus* Góczán et Juhász, species standing nearest to it.

Occurrence: Pénezskút Siltstone Formation, Upper Vraconian, dispar—Zone, bergeri-subzone.

A WORKING KEY TO THE SPECIES OF *PHYTOPHTHORA* DE BARY

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This paper presents a key for the identification of thirty-nine species of *Phytophthora*. They were divided into eight groups mainly on the basis of the ability to form sexual organs, surface morphology of the oogonium, type of antheridium and sporangial formation on solid media.

A new key for the identification of thirty-nine species of *Phytophthora* which are predominantly terrestrial is presented. Those species which are mostly aquatic and those known on the host alone were not included. This work combines the information derived from other schemes (FREZZI 1950, WATERHOUSE 1963, NEWHOOK et al. 1978, HO 1981, SHEPHERD et al. 1984) original descriptions of taxa and author's experience. It was found to be practical and therefore worth reporting.

a) List of species

The species of *Phytophthora* of the present key are listed hereunder.

1. *Phytophthora arecae* (Coleman) Pethybridge
2. *P. boehmeriae* Sawada
3. *P. botryosa* Chee
4. *P. cactorum* (Lebert & Cohn) Schroeter
5. *P. cajani* Amin, Baldev, and Williams
6. *P. cambivora* (Petri) Buisman
7. *P. capsici* Leonian
8. *P. cinnamomi* Rands
9. *P. citricola* Sawada
10. *P. citrophthora* (Smith & Smith) Leonian
11. *P. colocasiae* Raciborski
12. *P. cryptogea* Pethybridge & Lafferty
13. *P. drechsleri* Tucker
14. *P. erythroseptica* Pethybridge
15. *P. fragariae* Hickman
16. *P. heveae* Thompson
17. *P. hibernalis* Carne
18. *P. ilicis* Buddenhagen & Young
19. *P. infestans* (Montagne) de Bary
20. *P. inflata* Caroselli & Tucker
21. *P. iranica* Ershad
22. *P. insolita* Ann & Ko
23. *P. katsurae* Ko & Chang
24. *P. lateralis* Tucker & Milbrath

25. *P. meadii* McRae
26. *P. megakarya* Brasier & Griffin
27. *P. megasperma* Drechsler, sensu, Tompkins et al.
28. *P. mexicana* Hotson & Hartge
29. *P. MF4* Brasier & Griffin
30. *P. nicotianae* Breda de Haan
31. *P. palmivora* (Butler) Butler, sensu, Brasier & Griffin
32. *P. phaseoli* Thaxter
33. *P. porri* Foister
34. *P. primulae* Tomlinson
35. *P. quininea* Crandall
36. *P. richardiae* Buisman
37. *P. sinensis* Yongnian & Wenying
38. *P. syringae* (Klebahn) Klebahn
39. *P. vignae* Purss

b) Criteria and experimental conditions required for the use of the key

The above species of *Phytophthora* were subdivided into eight groups on the basis of (a) ability to produce sexual organs, (b) type of antheridium, (c) morphology of the oogonium wall and/or (d) ability to produce sporangia directly on solid media. Other characters taken into consideration for further differentiation were the average size of the papilla and the exit pore of the sporangium, the presence of swellings and chlamydospores, their size and position on the bearing hypha and the cardinal temperatures.

With regard to the conditions of experiment required in the use of the present key, the following needs to be pointed out. The scheme is based on organic rather than chemically defined cultural media. With the exception of HOHL (1975) who reported on the growth of sixteen species on a synthetic medium, the majority of investigators usually used organic media. Therefore a range of conditions as wide as possible is suggested, including different temperatures of incubation (15 to 35 °C) and the use of media such as cornmeal agar, Difco (17 g/lt), carrot agar (PETRI 1925, BRASIER 1972), CAMPBELL V-8 juice agar according to MILLER (1955), ZENTMYER et al. (1974), GERRETSON-CORNELL (1980) and lima bean agar, Difco (23 g/lt). For the study of sporangial formation the technique of CHEN and ZENTMYER (1970) and the cotyledon method (GERRETSON-CORNELL 1980) are recommended. Finally cultures of the isolate(s) to be identified should be examined every 2–3 days over a period of 2–4 weeks. Both the top and the bottom of the culture in the Petri dish should be examined, since it was observed that production of oogonia on V-8 agar by an isolate of *P. citricola* occurred only on the bottom of the plate (GERRETSON-CORNELL 1982).

KEY

GROUP 1: Homothallic, oogonium wall smooth, antheridium paragynous (a few amphigynous antheridia may be occasionally observed in *P. syringae* and *P. citricola*).

(A) Sporangia produced on solid media, either profusely or sparsely.

(a₁) Sporangia usually papillate ($\geq 3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$).

Antheridium sometime obscured in a knot of hyphae. Chlamydospores occasionally formed. No hyphal swellings. *P. cactorum*

- (a₂) Sporangia sparse, non papillate ($<3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$). Chlamydospores not formed.
- (a_{2,1}) Intercalary hyphal swellings rare in some isolates, common in others. A few amphigynous antheridia may be observed in some isolates. *P. syringae*
- (a_{2,2}) Hyphal swellings may be produced. Sporangia very variable in shape and size ($\bar{x} = 75\text{--}80 \mu\text{m}$), often compound and even in chains. *P. primulae*
- (B) Sporangia not formed or rare on solid media, profuse in liquid,* non papillate ($<3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$). No chlamydospores formed.
- (b₁) Antheridium inflated, lobed or branched, often twisting around the oogonial stalk, average length over $20 \mu\text{m}$. *P. inflata*
- (b₂) Antheridium not as above. A few amphigynous antheridia may be observed in some isolates. Sympodial branching of the sporophore usually at some distance from the base of the sporangium. Swellings often at points of branching of the sporangiophore. *P. citricola*
- (C) Sporangia not formed on solid media, profuse in liquid, non papillate ($<3.5 \mu\text{m}$), exit pore wide ($>7 \mu\text{m}$). Chlamydospores abundantly formed, terminal and/or intercalary or even double stalked in appearance, usually large ($\bar{x} > 45 \mu\text{m}$).
- (c₁) No growth above 26°C , optimum below 20°C . *P. lateralis*
- (c₂) Optimum temperature for growth $20\text{--}25^\circ\text{C}$, maximum $30\text{--}35^\circ\text{C}$. *P. quininea*

GROUP 2: Homothallic, oogonium wall smooth, antheridium amphigynous.

- (A) Sporangia produced on solid media, exit pore narrow ($\leq 7 \mu\text{m}$).
- (a₁) Sporangia papillate ($\geq 3.5 \mu\text{m}$), chlamydospores frequently observed, terminal. Intercalary hyphal swellings occasionally produced. *P. boehmeriae*
- (a₂) Sporangia non papillate ($<3.5 \mu\text{m}$); chlamydospores not formed or rare.
- (a_{2,1}) Sporangiophore inflated, often with a swelling below each sporangium. Slow growing. Optimum growth temperature below 20°C , maximum 30°C . *P. phaseoli*
- (a_{2,2}) Optimum growth at 20°C , no growth above $25\text{--}27^\circ\text{C}$. *P. ilicis*
- (a₃) Sporangia usually papillate ($\geq 3.5 \mu\text{m}$); chlamydospores not formed. Antheridium very small (c. $9 \times 11 \mu\text{m}$). *P. heveae*

* I.e. on agar media flooded with liquid.

- (B) Sporangia not formed on solid media but only in liquid; non papillate ($<3.5 \mu\text{m}$), exit pore usually wide ($>7 \mu\text{m}$). Chlamydospores not formed.
- (b₁) Terminal and/or intercalary hyphal swellings with tube like projections abundant at 15–20 °C. Optimum temperature for sporangia production 30 °C. *P. cajani*



Fig. 1. *P. erythroseptica*: Oogonium with three antheridia. $\times 710$

- (b₂) Intercalary hyphal swellings abundant, especially in liquid, at 24–26 °C. Occasionally two or three antheridia to a single oogonium may be observed (Fig. 1). *P. erythroseptica*
- (b₃) Hyphal swellings terminal or sessile. Optimum growth at 29 to 32 °C. Antheridium occasionally bicellular. *P. sinensis*
- (b₄) Hyphal swellings terminal or intercalary. Optimum growth temperature around 30 °C. *P. vignae*

GROUP 3: Homothallic, oogonium wall smooth, antheridium paragynous and amphigynous in variable proportions.

(A) Sporangia formed on solid media.

- (a₁) Sporangia terminal or intercalary, non papillate ($<3.5 \mu\text{m}$). Intercalary hyphal swellings formed. Chlamydospores not formed or rare. Oogonium wall sometimes unevenly thickened. Occasionally, two antheridia to a single oogonium. *P. porri*

- (a₂) Sporangia non papillate ($<3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$). Antheridium predominantly amphigynous in fresh cultures, predominantly paragynous in older cultures. Chlamydospores not formed. Optimum growth below 20°C , no growth above 25°C .

P. hibernalis

- (a₃) Sporangia papillate ($\geq 3.5 \mu\text{m}$). Chlamydospores formed sparsely, terminal or intercalary, c. $30 \mu\text{m}$ in diameter. Two antheridia to a single oogonium may be observed.

P. iranica

- (B) Sporangia not formed or rare on solid media, profuse in liquid. Non papillate ($<3.5 \mu\text{m}$), exit pore wide ($>7 \mu\text{m}$).

- (b₁) Antheridium predominantly paragynous. Chlamydospores not formed or rare (intercalary). Intercalary and terminal swellings formed.

P. megasperma

- (b₂) Antheridium predominantly amphigynous, chlamydospores not formed.

- (b_{2,1}) Sporangia large ($\bar{x} = 57 \times 36 \mu\text{m}$). Antheridium occasionally provided with a short hyphal projection.

P. fragariae

- (b_{2,2}) Intercalary swellings abundant, especially in water. Sporangia very variable in shape. Antheridia with a hyphal protuberance may be observed.

P. richardiae

GROUP 4: Homothallic, oogonium wall smooth, antheridium unknown. Sporangia not formed directly on solid media but profuse in liquid. Hyphal swellings and chlamydospores formed. Optimum growth at 32°C .

P. insolita

GROUP 5: Homothallic, antheridium amphigynous, oogonial wall verrucose (Fig. 2).

P. katsurae

GROUP 6: Heterothallic, oogonium wall smooth, antheridium amphigynous, sporangia profuse on solid media.

- (A) Chlamydospores not formed. Sporangia non papillate ($<3.5 \mu\text{m}$). Sporangiphore branched in a monochasial sympodium with a swelling below each sporangium.

P. infestans

- (B) Chlamydospores sparse or in some isolates only, terminal or intercalary, av. $30 \mu\text{m}$ diameter.

- (b₁) Sporangia papillate ($\geq 3.5 \mu\text{m}$), occasionally with two or three papillae, mostly obpyriform, partially deciduous, average pedicel $12 \mu\text{m}$ long.

P. megakarya

- (b₂) Sporangia papillate ($\geq 3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$), deciduous, average pedicel over $20 \mu\text{m}$ in length.

P. MF4

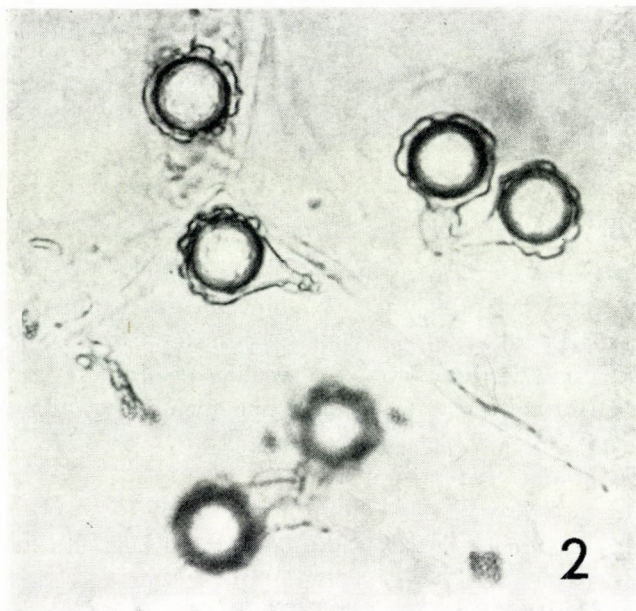


Fig. 2. *P. katsurae*: Oogonia with "verrucose" wall. $\times 770$

(C) Chlamydospores usually abundant.

(c₁) Sporangia terminal or intercalary, papillate ($\geq 3.5 \mu\text{m}$), often with two or three papillae, exit pore narrow ($\leq 7 \mu\text{m}$), vacuolate. Sporangiophore simple or irregularly branched, often with swellings at branching points. Chlamydospores profusely formed by some isolates, a few or not by others. Oogonia rarely observed in intra and interspecific pairings.

P. citrophthora

(c₂) Chlamydospores hyaline to golden brown, mean up to $40 \mu\text{m}$ diameter. Sporangia papillate ($\geq 3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$), deciduous, often with two or three papillae.

P. palmivora

GROUP 7: Heterothallic, antheridium amphigynous. Sporangia usually* not formed on solid media, profuse in liquid, non papillate ($< 3.5 \mu\text{m}$), exit pore wide ($> 7 \mu\text{m}$).

(A) Mycelium typically coralloid on corn meal agar. "Botryose" swellings and chlamydospores produced on various media. Oogonium wall smooth, antheridium unicellular and/or bicellular.

P. cinnamomi

(B) Neither botryose swellings nor chlamydospores formed.

(b₁) Intercalary hyphal swellings abundant in culture or in liquid. Oogonium wall smooth.

P. cryptogea, *P. drechsleri*

* A few sporangia may be formed on solid media by *P. cryptogea* and *P. drechsleri*.

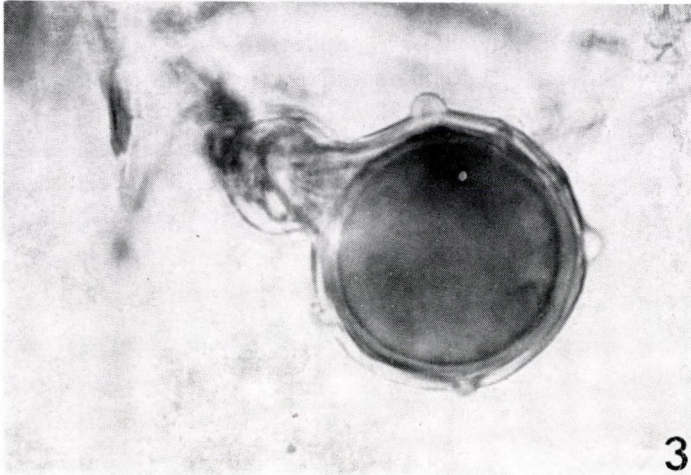


Fig. 3. *P. cambivora*: "Bullate" oogonium. $\times 1500$

- (b₂) Intercalary hyphal swellings may be formed in liquid. Oogonium wall "bullate" (Fig. 3) as well as smooth or undulate. Antheridium unicellular and/or bicellular. *P. cambivora*

GROUP 8: Both heterothallic and homothallic isolates observed, oogonium wall smooth to wrinkled, antheridium amphigynous. Sporangia formed on solid media.

- (A) Prevalently homothallic, oogonium wall smooth. Sporangia deciduous, non papillate ($< 3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$); branching of the sporophore sympodial to irregular with (often) a swelling at branching point. Chlamydospores usually formed ($\bar{x} = c. 27 \mu\text{m}$), terminal or intercalary. *P. colocasiae*

- (B) Prevalently heterothallic.

- (b₁) Oogonium wall smooth.

- (b_{1,1}) Sporangia in conspicuous, yellowish-brownish clumps, deciduous, non papillate ($< 3.5 \mu\text{m}$). Chlamydospores rare, av. $19 \mu\text{m}$ diameter. *P. botryosa*

- (b_{1,2}) Sporangia very irregular in shape, papillate ($\geq 3.5 \mu\text{m}$), often with two papillae, exit pore narrow ($\leq 7 \mu\text{m}$), non deciduous. Chlamydospores sparse, intercalary, av. $33 \mu\text{m}$ diameter. *P. mexicana*

- (b_{1,3}) Sporangia terminal, occasionally intercalary, papillate ($\geq 3.5 \mu\text{m}$), exit pore narrow ($\leq 7 \mu\text{m}$), deciduous. Chlamydospores not formed or rare, av. $32 \mu\text{m}$ diameter. *P. arecae*

- (b₂) Oogonium wall smooth or wrinkled.
- (b_{2,1}) Sporangia deciduous, papillate ($\geq 3.5 \mu\text{m}$) or not, sometimes bipapillate, exit pore narrow ($\leq 7 \mu\text{m}$). Chlamydospores not formed or rare, av. $32 \mu\text{m}$ diameter. *P. meadii*
- (b_{2,2}) Sporangia not always formed or sparse on solid media, very variable and irregular in shape, often vacuolated, usually papillate ($\geq 3.5 \mu\text{m}$), often with two or three papillae. Chlamydospores not formed or rare. *P. capsici*
- (b_{2,3}) Sporangia terminal or intercalary, rounded at base, papillate ($\geq 3.5 \mu\text{m}$) or not, exit pore narrow ($\leq 7 \mu\text{m}$). Chlamydospores often formed, terminal or intercalary, av. $30\text{--}35 \mu\text{m}$ diameter. *P. nicotianae*

Discussion

The present study has highlighted the difficulty involved in classifying the genus *Phytophthora*. This is mainly because of the high variability exhibited by certain species with regard to certain characteristics. For example, *P. nicotianae* that this writer always found to be heterothallic on V-8 agar and cornmeal agar, often produced abundant oogonia on lima bean agar. *P. katsuriae* yielded abundant sporangia on SCHMITTHENNER's medium (ROBERTSON 1980), at 23°C but did not form them directly on various other media (GERRETTSON-CORNELL: unpublished data). Again the presence of intercalary swellings in clusters of the net like type of configuration was observed in cultures of *P. boehmeriae* on V-8 agar whereas on cornmeal agar these were not formed (GERRETTSON-CORNELL: unpublished data). These facts emphasize the necessity of using a wide range of experimental conditions in the identification of an unknown isolate of *Phytophthora*.

On the basis of the criteria for identification adopted it was not possible to separate *P. cryptogea* from *P. drechsleri*. This concurs with the findings of BUMBIERIS (1974). The mycelium of *P. drechsleri* appears to be formed of regular, tubular hyphae, whereas *P. cryptogea* can also be entirely coralloid. On the other hand, *P. cryptogea* may present a wide range of mycelia, closer to either species. Both *P. cryptogea* and *P. drechsleri* but especially the latter may form some sporangia directly on solid media. However, the frequency of occurrence of this phenomenon is erratic. Again, *P. drechsleri* usually shows marked sporangial polymorphism. Yet, the same shapes may be found in *P. cryptogea* and there are isolates of this species which cannot be distinguished from *P. drechsleri* on the basis of this characteristic. With regard to the ability to grow at $34\text{--}36^\circ\text{C}$ that *P. drechsleri* is supposed to show (WATERHOUSE 1963), it has proved to be too variable to be of any taxonomic value. Similarly

the widening of the sporangiophore below the sporangium, although more often observed in *P. drechsleri* than in *P. cryptogea*, is not a constant feature and is therefore of no value in identification. No real differences also exist in the sexual organs of these two fungi, nor in the intercalary swellings and other minor morphological characteristics.

Finally in this study, the term *P. nicotianae* Breda de Haan sensu Shepherd (SHEPHERD et al. 1984) has been used to include the two varieties *P. nicotianae* var. *nicotianae* and *P. nicotianae* var. *parasitica* as a matter of priority and simplicity.

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SEVEN SPECIES OF USTILAGINALES, NEW FOR HUNGARY

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The following seven species of Ustilaginales are reported for the first time from Hungary: *Anthracoidea intercedens* on *Carex lasiocarpa*, *Tilletia olida* on *Brachypodium pinnatum*, *Urocystis agrostidis* on *Agrostis capillaris*, *U. alopecuri* on *Alopecurus pratensis*, *U. ulei* on *Festuca heterophylla* and *F. valesiaca*, *Ustilago thlaspeos* on *Arabis hirsuta*, and *Ustilentyloma fluitans* on *Glyceria fluitans*.

The progresses in the knowledge of Hungarian Ustilaginales were synthesized by MOESZ (1950), UBRIZSY (1967), and VÁNKY, GÖNCZÖL and TÓTH (1982). Through systematic collections, during the last years, several smut species were recorded as new for Hungary. At present IMRE (in press) is publishing *Entyloma linariae* on *Linaria vulgaris*. Furthermore, the following seven species have been recorded: 1. *Anthracoidea intercedens* in ovaries of *Carex lasiocarpa*, 2. *Tilletia olida* in leaves of *Brachypodium pinnatum*, 3. *Urocystis agrostidis* in leaves and culms of *Agrostis capillaris*, 4. *U. alopecuri* in leaves and culms of *Alopecurus pratensis*, 5. *U. ulei* in leaves of *Festuca heterophylla* and *F. valesiaca*, 6. *Ustilago thlaspeos* in seeds of *Arabis hirsuta*, and 7. *Ustilentyloma fluitans* in leaves of *Glyceria fluitans*.

On *Carex*, subgen. *Carex*, sectio *Paludosae* sensu lat. [treated by CHATER (1980) as four sections: *Carex*, *Paludosae*, *Pseudocyperae*, *Vesicariae*], there are four *Anthracoidea* species possessing small and echinate spores: *A. americana*, *A. inclusa*, *A. intercedens*, and *A. subinclusa*. According to NANNFELDT (1979: 9) they may be differentiated as follows:

- 1 Spore wall with very distinct internal swellings *A. americana*
- Spore wall without internal swellings 2
- 2 Spines stout, up to 2 μm high and apical flattenings up to 2 μm across, widely spaced and easily broken. Principal hosts *C. riparia* and *C. vesicaria* *A. subinclusa*
- Spines more slender and lower, more closely set, less easily broken ... 3
- 3 Spines often reaching 1.5 μm in height. Spore surface wrinkled between the spines. On *C. lasiocarpa* and its hybrids only *A. intercedens*
- Spines only rarely exceeding 1.0 μm in height. Spore surface smooth between the spines. Principal host *C. rostrata* *A. inclusa*

***Anthracoidea intercedens* Nannfeldt**Symb. Bot. Upsal. **22** (3): 22, 1979

Sori (Fig. 1) in scattered ovaries forming rounded, hard, black bodies of a few mm in diameter, first covered by a thin, silvery membrane of fungal origin, when ripe broken up

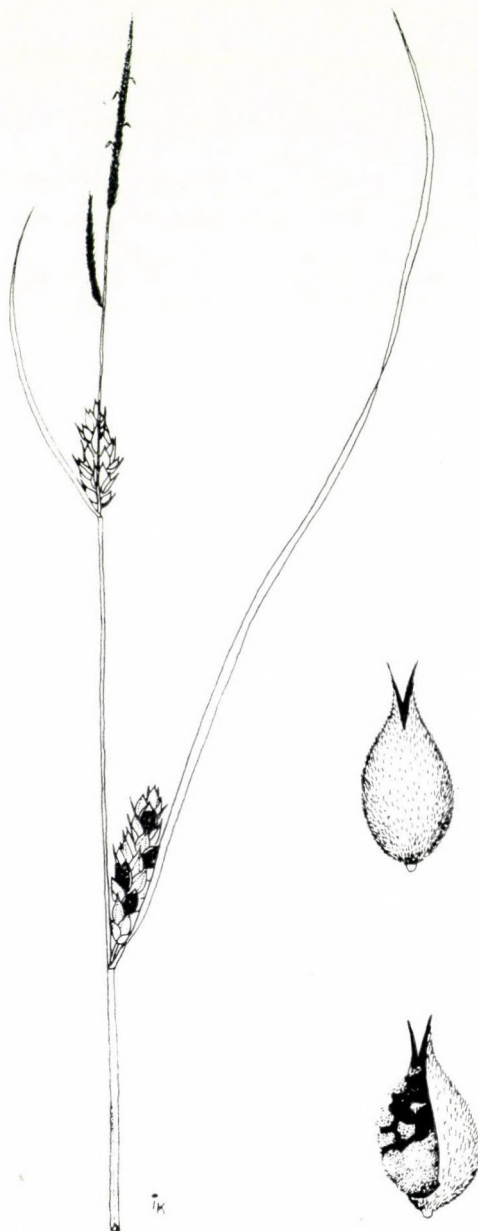


Fig. 1. *Anthracoidea intercedens* on *Carex lasiocarpa*

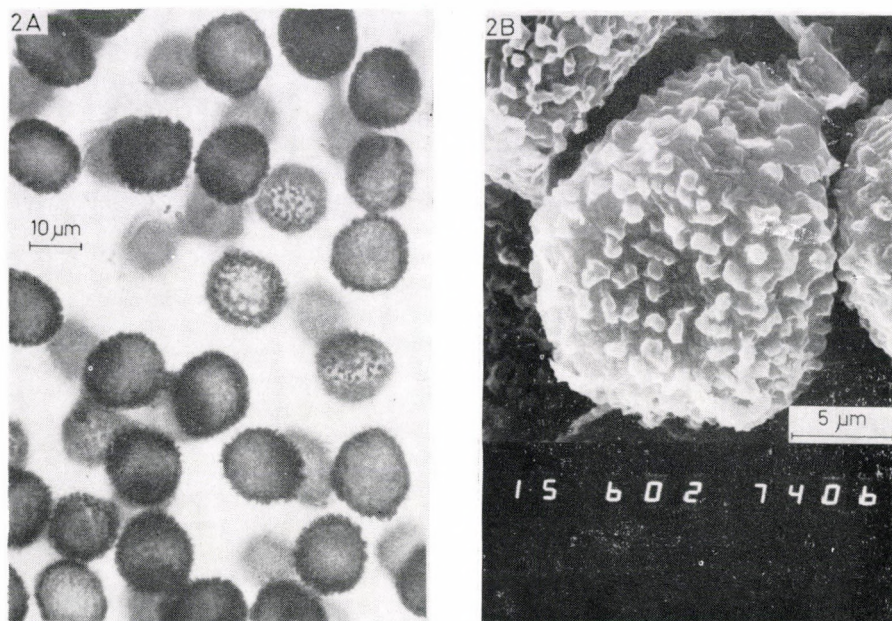


Fig. 2. Spores of *Anthracoidea intercedens*, A) in LM, B) in SEM

into small pieces formed by agglutinated spore masses. Spores (Fig. 2) globose, broadly ellipsoidal to moderately rounded-angular, $11\text{--}19 \times 13\text{--}23\text{ }\mu\text{m}$; wall $1\text{--}1.5\text{ }\mu\text{m}$ thick, without internal swellings; surface echinulate and striate-rugulose between the spines. Spines $1\text{--}1.5\text{ }\mu\text{m}$ high, apically flattened and slightly enlarged, irregularly, rather closely spaced, more easily detaching than in *A. subinclusa*.

It is known on *Carex lasiocarpa* Ehr. and its hybrids from Europe (England?, Finland, Norway, Polen?, Soviet Union, Sweden), on *C. rhynchophysa* Fischer, C. A. Meyer & Avé-Lall, and on *C. sordida* Cham. ex van Heurck & Muell. from Asia (Soviet Union).

In Hungary it was collected on *C. lasiocarpa*, Somogy Co, near the village Darány, 14. VI. 1983, coll. Gy. SZOLLÁT (BP, HUV).

There are c. 100 known species of *Tilletia*. The great majority of them develop their sori in the ovaries of Gramineae, but a few species have sori in the leaves and culms forming streaks. Such a species, *T. olida*, was recently discovered in Hungary.

Tilletia olida (Riess) Schröter

in COHN, Beiträge zur Biologie der Pflanzen 2: 366, 1877

Sori (Fig. 3) in leaves and culms forming long striae between the veins, often confluent, at first lead-coloured, later brown. With age the epidermis split up, the blades fray and shred, and the blackish-brown, agglutinated to semi-powdery, foetid spore mass becomes exposed. Attacked shoots are usually shortened and sterile. Spores (Fig. 4) globose to ovoid, $18\text{--}24 \times 19\text{--}25\text{ }\mu\text{m}$, light golden-brown; wall $0.5\text{--}1\text{--}(2)\text{ }\mu\text{m}$ thick, reticulate to cerebriform, 7–10 meshes



Fig. 3. *Tilletia olida* on *Brachypodium pinnatum*

per spore diameter, areolae $0.5\text{--}1.5\ \mu\text{m}$ high and $1.5\text{--}2.5\ \mu\text{m}$ wide. Sterile cells among the spores few, round and smaller than the spores ($11\text{--}18\ \mu\text{m}$), hyaline to yellowish tinted, smooth. Germination (ZOGG 1967) by unseptate promycelium apically bearing four elongated basidiospores.

It is known on *Brachypodium* spp. from Europe and Asia. In Hungary it was found on *Brachypodium pinnatum* (L.) Beauv., Heves Co, near the village Kerecsend, "Kerecsendi erdő", 160 m, 12. VI. 1981, coll. J. GÖNCZÖL and Á. RÉVAY (BP, HUV).

The species of *Urocystis* are characterized by persistent spore balls formed of one to numerous, dark, fertile spores, surrounded by a few to numerous, paler sterile cells. The delimitation of the species is often difficult because of the scanty morphological characters of the spore balls, spores and

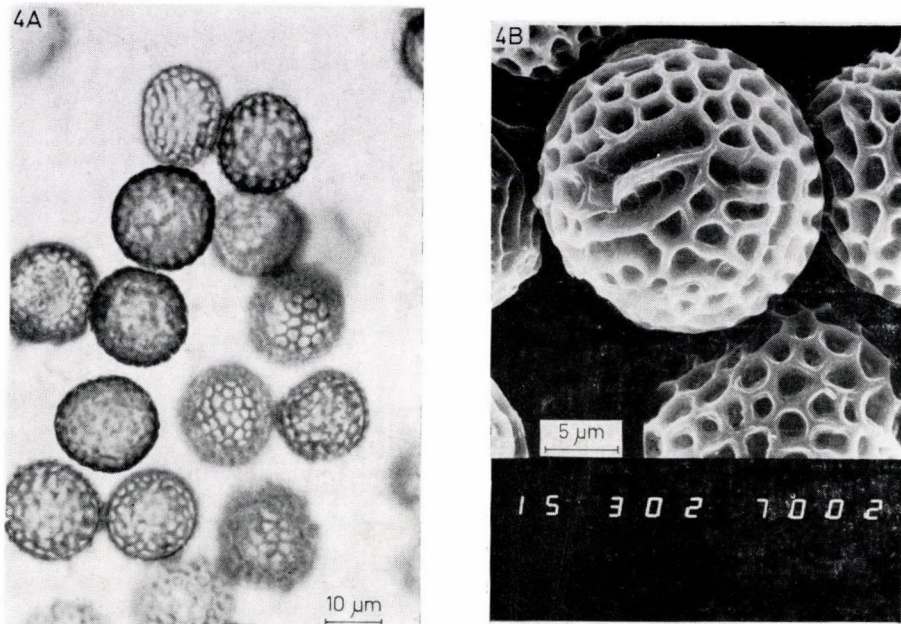


Fig. 4. Spores of *Tilletia olida*, A) in LM, B) in SEM

sterile cells. The number of spores in the balls, the colour and size of the balls, spores and sterile cells, the thickness of the spore wall, as well as the continuity or discontinuity of the outer layer of sterile cells are useful characters but often interfluent.

***Urocystis agrostidis* (Lavrov) Zundel**

The Ustilaginales of the World. — Pennsylvania State Coll. School. Agric. Dept. Bot. Contrib. **176**: 307, 1953

Sori (Fig. 5) in leaves, sheaths and culms forming long streaks between the veins, at first lead-coloured and covered by the epidermis which ruptures longitudinally and the black, powdery mass of spore balls become scattered. *Spore balls* (Fig. 6) globose to ovoid, 20–36 (–40) × 24–48 (–56) µm in diameter, composed of 1–4 (–5) central spores and a continuous layer of peripheral sterile cells. *Spores* subglobose to ovoid, 12–16 × 13.5–20 µm, olivaceous-brown, smooth. *Sterile cells* subglobose, ovoid or hemispherical, 5–14 µm long, light yellowish-brown, smooth.

On different *Agrostis* species in Europe, Asia, N. and S. America. In Hungary it is reported on *Agrostis capillaris* L. (= *A. tenuis* Sibth.), Heves Co, Mts. Bükk, near the village Szilvászvárad, 700 m, 24. VI. 1982, coll. J. GÖNCZÖL, Á. RÉVAY, S. TÓTH and K. VÁNKY (BP, HUV).



Fig. 5. *Urocystis agrostidis* on *Agrostis capillaris*



Fig. 6. Spore balls of *Urocystis agrostidis*

***Urocystis alopecuri* Frank**

Die Krankheiten der Pflanzen, Pilze. Breslau. 1880: 440.

It resembles *Urocystis agrostidis* (Fig. 7). *Spore balls* (Fig. 8) globose to ovoid, 15-35 (-42) μm long, composed of 1-3 central spores mostly completely surrounded by sterile cells. *Spores* globose, ovoid or rounded polyangular, 12-17 \times 14.5-20 μm , light brown; wall smooth, c. 0.8 μm thick. *Sterile cells* globose, ovoid to irregular, 6.5-12 μm long, smooth, yellow.

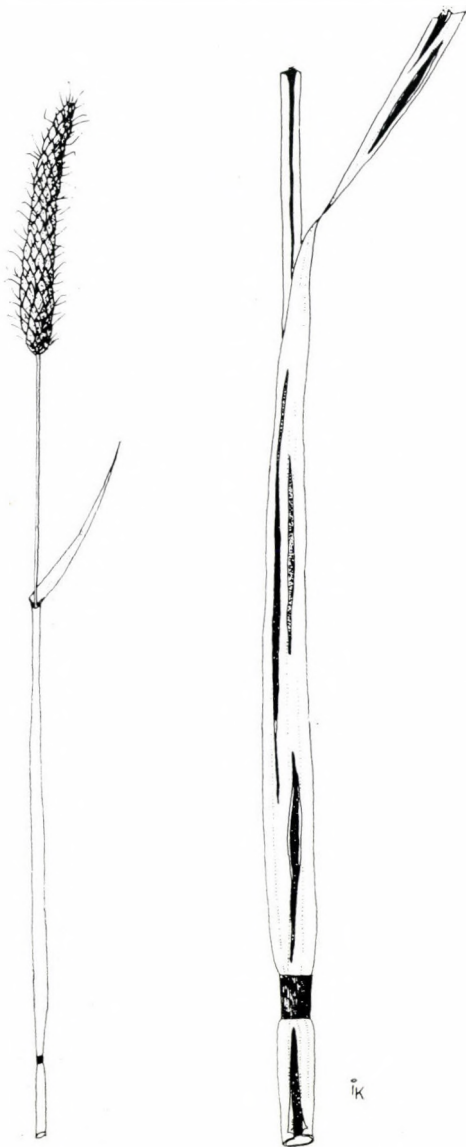


Fig. 7. *Urocystis alopecuri* on *Alopecurus pratensis*

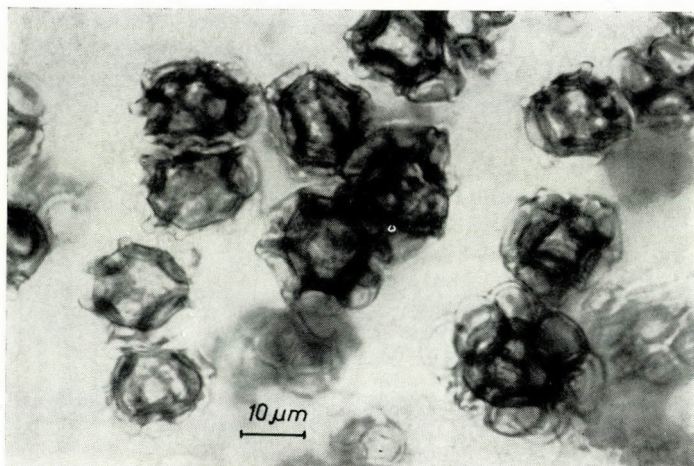


Fig. 8. Spore balls of *Urocystis alopecuri*

On *Alopecurus* spp. in Europe and Asia. In Hungary it was collected on *A. pratensis* L., Pest Co, Mts. Börzsöny, mountain Kelemen-hegy near the village Kóspallag, 300 m, 1. VI. 1983, coll. Á. RÉVAY and J. GÖNCZÖL (BP, HUV).

Urocystis ulei P. Magnus

Bot. Zeitung **36**: 119, 1878

Syn. *Ustilago macrospora* Desm. (nomen ambiguum).

It appears like other *Urocystis* species on Gramineae, forming streaks on the leaves (Fig. 9). *Spore balls* (Fig. 10) globose, ovoid to slightly irregular, $23-33 \times 25-40 \mu\text{m}$, composed of 1-2 (-3) spores surrounded by a well-developed, continuous, or nearly continuous layer of sterile cells. *Spores* globose, ovoid, rounded polyangularly irregular to elongated, $11-14.5 \times 13-19 \mu\text{m}$, dark reddish-brown, smooth. *Sterile cells* globose, ovoid to elongated, $5-6 \times 6-14 \mu\text{m}$, yellow to light yellowish-brown, thick-walled (c. $2 \mu\text{m}$) on the contact sides, thinner on the free surface which collapses with age giving a ridged appearance to the spore ball profile.

On *Festuca* spp. in Europe, Asia and N. America. In Hungary it was found on *Festuca heterophylla* Lam., Komárom Co, Mts. Pilis, "Kis Vadállókövek", 475 m, 23. VI. 1983, coll. K. VÁNKY (BP, HUV). On *F. valesiaca* Schleicher ex Gaudin, Borsod-Abauj-Zemplén Co, Mts. Bükk, "Nagymező", 790 m, 25. VI. 1982, coll. J. GÖNCZÖL, Á. RÉVAY, S. TÓTH and K. VÁNKY (BP, HUV).

The smut genus most rich in species is *Ustilago* (over 350 species). It is characterized by sori in various parts of the hosts, at maturity bursting and exposing usually powdery, blackish-brown, purplish-brown, olivaceous, more seldom pale spore mass; spores single; sterile cells absent. Spore germination by means of septate promycelium bearing basidiospores (sporidia) or infection threads laterally and terminally.

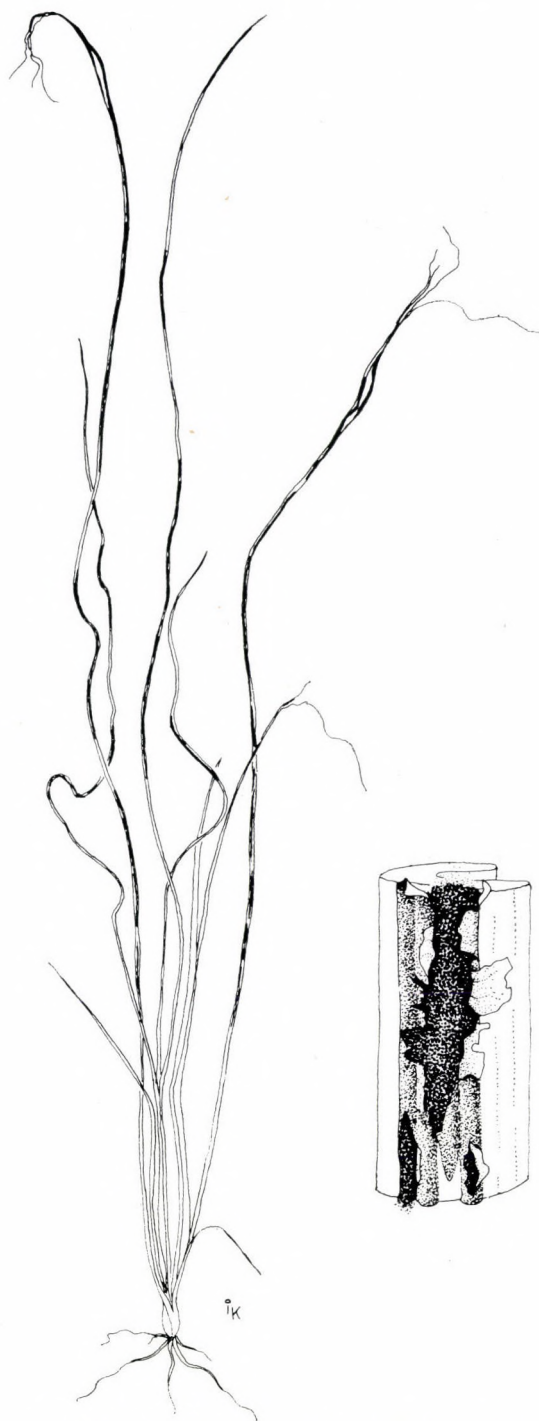


Fig. 9. *Urocystis ulei* on *Festuca heterophylla*

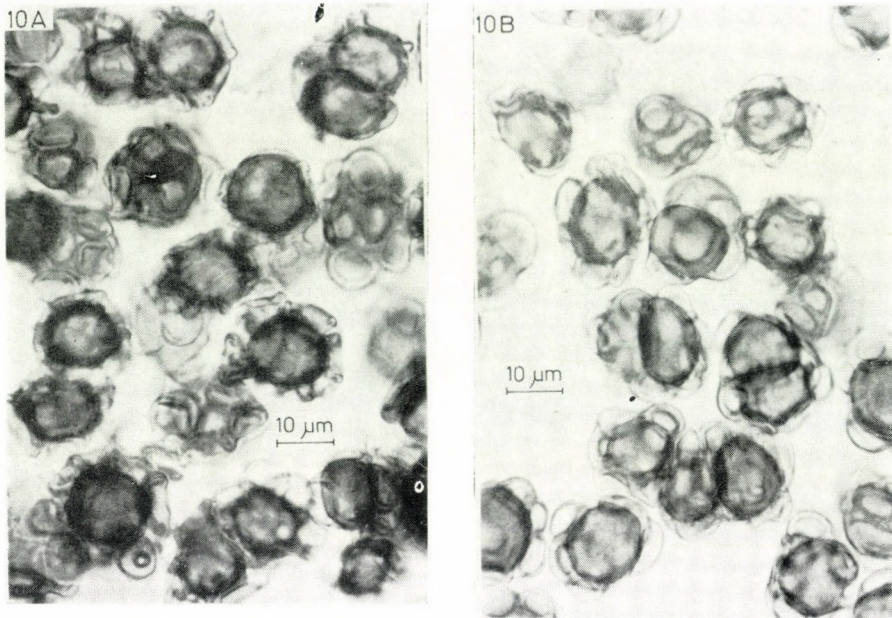


Fig. 10. Spore balls of *Urocystis ulei*, A) on *Festuca heterophylla*, B) on *F. valesiaca*

***Ustilago thlaspeos* (G. Beck) Lagerheim**

in Sydow, *Ustilaginales* No. 118, 1897

Sori (Fig. 11) replacing the seeds, light brown, dusty, liberated when the siliquae are opened, rather inconspicuous. *Spores* (Fig. 12) globose, subglobose, ovoid to irregularly round, $10\text{--}16 \times 11\text{--}18\text{ }\mu\text{m}$, yellow to light yellowish-brown, provided with moderately densely situated, irregular, $0.5\text{--}1\text{ }\mu\text{m}$ high warts which on one side of the spore are more dense and elongated ($1.5\text{--}2.5\text{ }\mu\text{m}$); in SEM even finely and densely verruculose between the irregular warts or tubercles. Infection systemic.

On different Cruciferae: *Arabis*, *Cardamine*, *Cardaminopsis*, *Draba*, *Thlaspi* spp. in Europe. In Hungary it was discovered on *Arabis hirsuta* (L.) Scop., Pest Co, Mt. Huszonnégyszökrös-hegy near the town Budaörs, 280 m, 29. X. 1982, coll. S. TÓTH (BP, HUV).

The genus *Ustilentyloma* was erected by SAVILE (in SAVILE and PARMELEE 1964: 708). *Sori* forming leaf spots. *Spores* single or in more or less compact groups, embedded in the host tissue. Resembles *Entyloma* but germination is of *Ustilago* and not *Tilletia* type. Only two species are known, both on Gramineae: *Ustilentyloma pleuropogonis* Savile, on *Pleuropogon sabinei* R. Br. from Canada, and *U. fluitans* on different *Glyceria* species from Europe.

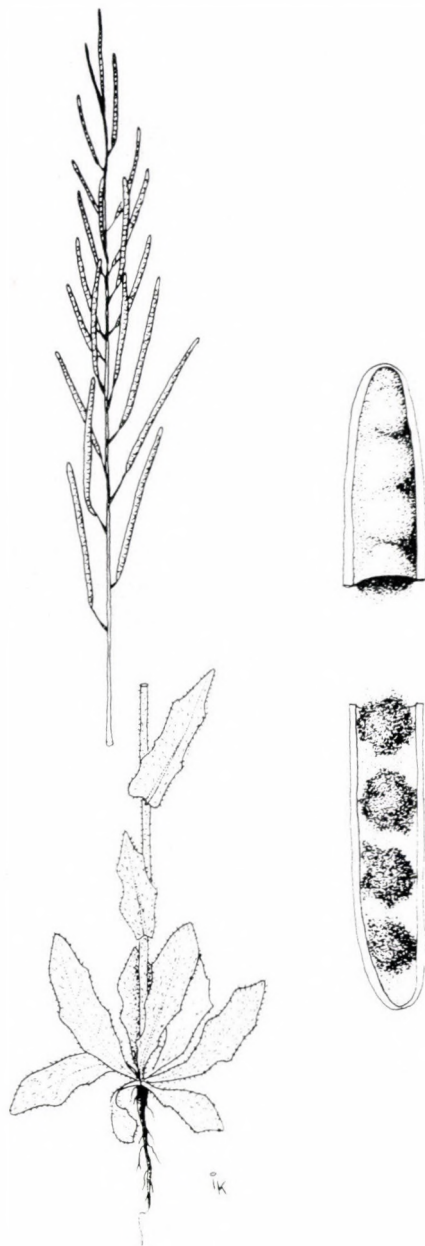


Fig. 11. *Ustilago thlaspeos* on *Arabis hirsuta*

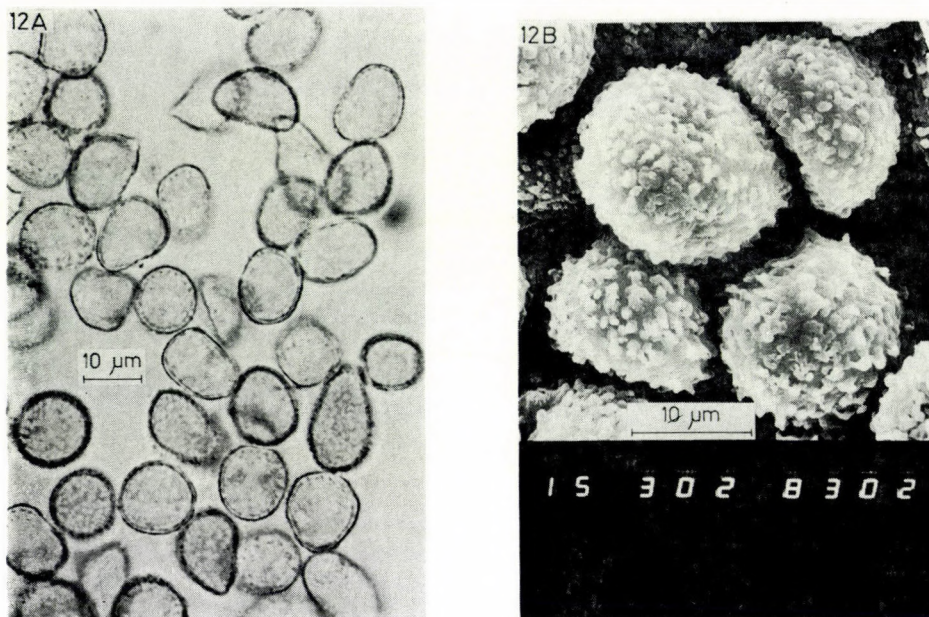


Fig. 12. Spores of *Ustilago thlaspeos*, A) in LM, B) in SEM

***Ustilentyloma fluitans* (Liro) Vánky**

Microbiol. Bucuresti 1: 328, 1970

Sori (Fig. 13) in leaves and leaf-sheaths, forming at first light yellow, later dark brown, linear or ovoid, 1–10 mm long spots, often confluent, usually with indefinite or lighter coloured margins. *Spores* (Fig. 14) globose, ovoid or slightly angularly irregular, (7.5–) 8.5–14 × 8.5–15.5 (–17) μm , hyaline or light yellow, with smooth, c. 1 μm thick wall. Germination in situ, in the leaves immersed in water, by means of a four-celled promycelium on which the basidiospores are produced laterally and terminally. *Basidiospores* ovoid, hyaline, 2–3 × 8–12 μm , 2 and 2 copulate by a thin, short copulating-bridge (VÁNKY 1970: 329).

Known hitherto on *Glyceria fluitans* (L.) R. Br. (Finland, Romania and Sweden), and on *G. plicata* Fr. (Czechoslovakia and Romania). In Hungary it was collected on *G. fluitans*, Vas Co, near the village Szőce, 180 m, 22. VI. 1983, coll. J. GÖNCZÖL, K. IMRE, S. TÓTH and K. VÁNKY (BP, HUV).



Fig. 13. *Ustilentyloma fluitans* on *Glyceria fluitans*

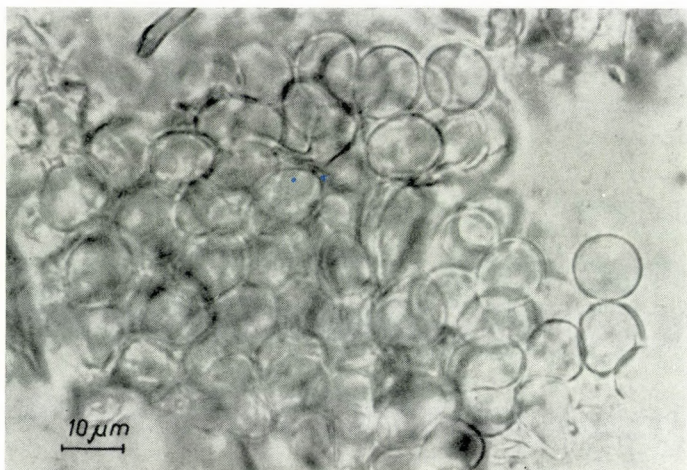


Fig. 14. Spores of *Ustilentyloma fluitans*

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We express our sincere thanks to Ms. Maria Heldt for revision of the English of the manuscript.

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EAST AFRICAN BRYOPHYTES, VII

THE HEPATICAE OF THE USAMBARA RAIN FOREST PROJECT EXPEDITION, 1982*

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From the 94 liverwort species listed 72 were collected in the Usambara, 42 in the Uluguru Mts. 18 taxa proved to be new for the Usambaras while only 2 for the Ulugurus. Four of them are new for the whole of East Africa, as *Pycnolejeunea angustiflora* Steph., *Lejeunea flavovirens* J. Ångstr., *Cololejeunea apiculata* (E. W. Jones) Schust. and *Cololejeunea elegans* Steph. *Cladolejeunea aberrans* (Steph.) Zwickel was the first time collected since its description and its Massula type oil bodies are described. *Cololejeunea amaniensis* Pócs and *Cololejeunea tanneri* Pócs are described as new to science. A brief phytogeographical evaluation of species collected is added.

Keywords: Hepaticae, Usambara, Uluguru, Tanzania, Africa

Introduction

Within the frame of the preliminary works of the joint Tanzanian-Swedish-Hungarian Usambara Rain Forest Research Project in early 1982 Professors J. B. HALL, O. HEDBERG, A. BORHIDI and the Author paid a short visit in the East and West Usambara and in the Uluguru Mountains, accompanied by Mr. M. MSHOO, staff member of the Tanzanian Silvicultural Research Station (Lushoto). While the other participants concentrated on phanerogams, the Author collected the cryptogamic plants and in this paper gives an account on his determinations of the liverworts collected and adds a few records also from his previous gatherings. In the enumeration of the collected species the following abbreviations are used:

EUS: East Usambara Mts., WUS: West Usambara Mts., ULU: Uluguru Mts.

If the taxon is new for the flora of the area concerned, a sign of * stands before the abbreviation. The specimen numbers (after the geographical abbreviations and the indication of substrates) consist of a locality number and a letter according to the different species within the same locality. The localities and other collecting data are, as follows:

East Usambara Mountains

- 6946.** Amani West Forest Reserve behind the "Forest Houses" of Amani. Submontane rain forest rich in epiphyllous liverworts at 950 m alt. 19. Feb. 1982.
- 6947.** Amani-Sigi Forest Reserve at the Sigi headwaters area S of Amani, near Kwamkoro Sawmills. Submontane rain forest with rock outcrops at 900-1000 m alt., rich in epiphyllous liverworts. 19. Feb. 1982.
- 6948.** Bomole Hill 1.5 km W of Amani. Partly secondary submontane forests at 960-1060 m alt., no epiphyllous bryophytes. 20. Feb. 1982.

* Usambara Rain Forest Project Publications, No. 1.

West Usambara Mountains

- 6205.** Shume-Magamba Forest Reserve. "Kandele Kampala" saddle between Magamba and Mabweni summits. Montane rain forest at 1900 m alt. Coll. T., T. and S. Pócs, 23. June 1970.
- 6950.** Shume Nature Forest Reserve, mesic montane evergreen forest dominated by *Ocotea* and *Podocarpus*. No epiphyllous bryophytes. 21. Feb. 1982.
- 6951.** Dry, sclerophyllous forest on rock outcrops, with scattered *Juniperus excelsa* trees and with many succulents NE of Shume village, at 1850 m alt. 21. Feb. 1982.
- 6953.** Shume-Magamba Forest Reserve, N of Lushoto. NW slopes of Magamba summit. Mesic evergreen montane forest between 2000–2150 m alt. No epiphyllous liverworts. 21. Feb. 1982.
- 6954.** Shume-Magamba Forest Reserve, N of Lushoto. N summit of Magamba. Relatively dry elfin woodland without epiphyllous bryophytes, at 2200 m alt. 21. Feb. 1982.
- 6955.** Baga II Forest Reserve W of Mazumbai village. E slope of Kwagoroto above Kambi Falls, montane evergreen forest at 1750–1900 m. 23. Feb. 1982.
- 6956.** Baga II. Forest Reserve, E summit of Kwagoroto, ericaceous (*Philippia*) heath and dry elfin forest at 1900 m. 23. Feb. 1982.
- 6957.** Baga II. Forest Reserve, W of Mazumbai, E facing half shady cliff of siliciferous rocks at 1700–1900 m alt. 23. Feb. 1982.
- 6371.** Mazumbai, Dar es Salaam University Forest Reserve, E slope of Sagara ridge. Montane evergreen forest at 1600–1750 m alt. Coll. T. Pócs and E. W. JONES, 7. Jan. 1971.
- 6372.** and **6959.** Mazumbai, DSM University Forest Reserve. E slope of Sagara ridge at 1700–1850 m alt. Montane rain forest (partly mossy forest) rich in epiphytes. Coll. T. Pócs and E. W. JONES (6372). 7. Jan. 1971 and 23. Feb. 1982.
- 6960.** Mazumbai, DSM University Forest Reserve, on the sharp, rocky Sagara ridge at 1850–1980 m alt. Mosaic of mossy forest, elfin woodland and *Philippia* heath. 23. Feb. 1982.
- 6962.** Mazumbai, SE corner of DSM University Forest Reserve, submontane rain forest at 1400–1500 m alt. ("LUNDGREN's plot".) 24. Feb. 1982.
- 6963.** Mazumbai, S end of DSM University Forest Reserve, submontane rain forest at the W side of the road, in a damp gully, epiphylls on *Marattia fraxinea* fern leaflets, at 1550 m. Coll. T. Pócs and A. BORHIDI. 24. Feb. 1982.
- 6964.** Near Mgwashi village N of Mazumbai, on rocky earth banks, surrounded by dry thicket, at 1500 m. 24. Feb. 1982.

Uluguru Mountains S of Morogoro town

- 6966.** Mwere valley, very wet submontane rain forests at 1450–1500 m alt, with many epiphyllous liverworts especially on *Marattia* leaflets. 28. Feb. 1982.
- 6967.** W slope of Palata, montane rain forest at 1600–1800 m alt., rich in epiphyllous liverworts. 28. Feb. 1982.
- 6968.** Palata ridge, montane mossy forest and elfin forest at 1800–1900 m alt. 28. Feb. 1982.
- 6969.** The rocky N end of Palata ridge. *Philippia* heath at 1650 m alt. on granitic groundstone, with shallow, acidic soil. 28. Feb. 1982.
- 6970.** Rocky streambed right above Morningside, 1200–1300 m. 1. March 1982.
- 6971.** N slope of Bondwa, at the lower edge of the forest reserve above Morningside. Submontane rain forest at 1320 m alt. 1. March 1982.
- 6972.** E of Morningside, near the "Schlesien Mission", *Cupressus lusitanica* plantation at 1250 m alt. 1. March 1982.

After the localities a short indication of distribution is given or a reference to previous parts of the same East African Bryophytes ("EAB") series, where distributional records were given. The specimens enumerated are deposited in DSM, UPS and in VBI. The enumeration is followed by a short phytogeographical evaluation and by the description of two new species.

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LIST OF SPECIES COLLECTED

[Lepidoziaceae

1. *Lepidozia cupressina* (Sw.) Lindenb. ssp. *cupressina* — Corticolous. *WUS: 6954/E¹, 6954/J, 6960/O¹, 6960/Y. Afro-American species, distribution cf. GRADSTEIN, PÓCS, VÁÑA 1984: 159–160, new to the Usambaras.
2. *Bazzania borbonica* (Steph.) Steph. — Corticolous. ULU: 6967/L. Lemurian species, EAB I, III.
3. *Bazzania decrescens* (Lehm. et Lindenb.) Trev. — Corticolous. WUS: 6953/A, 6955/W, 6960/H; ULU: 6969/E. Widespread in tropical Africa and Australasia, EAB I, II, III.
4. *Bazzania nitida* (Web.) Grolle — Terricolous and corticolous. WUS: 6953/C, 6955/E, 6959/F, 6960/C, L; ULU: 6967/K. Widespread in the mountains of tropical and South Africa, in South America and Australia. EAB I, III.

Jungermanniaceae

5. *Chandonanthus hirtellus* (Web.) Mitt. — On half shady rocks. ULU: 6969/D. Palaeotropical species, EAB I, map by Pócs, 1971: 852.

Geocalycaceae

6. *Lophocolea concreta* Mont. — Terricolous and lignicolous. WUS: 6955/FA; *ULU: 6972/C. Widespread in tropical and in South Africa, EAB I, III, IV.
7. *Lophocolea fragrans* (Moris et De Not.) Gott., Lindenb. et Nees — Lignicolous. WUS: 6955/FC. Atlantic and S Europe, Africa, EAB I, III.
8. *Lophocolea lucida* (Spreng. ex Lehm.) Mont. — Lignicolous. WUS: 6962/B. Tropical African, EAB I, III.
9. *Lophocolea martiana* Nees — Lignicolous. WUS: 6955/FB. Tropical Afro-American, previously known in Africa under the synonym of *L. congoana* Steph., EAB III, see map in GRADSTEIN, PÓCS, VÁÑA 1984: 135.

¹ Forms approaching ssp. *pinnata* (Hook.) Pócs

Plagiochilaceae

10. **Plagiochila angustitexta** Steph. — On shady rocks. WUS: 6960/K. Known only from the West Usambara Mts. and seems to be endemic (cf. E. W. JONES 1962: 297).
11. **Plagiochila cambuena** Steph. — Corticolous. *WUS: 6950/E. Published only from Madagascar (cf. VANDEN BERGHEM 1981: 76), however, E. W. JONES identified many specimens of our joint collectings from the Southern Highlands of Tanzania, as *P. cambuena* Steph. (unpublished). New for the Usambaras.
12. **Plagiochila divergens** Steph. var. **myriocarpa** (Pears.) E. W. Jones — Epiphyllous. ULU: 6966/BA. Widespread afromontane species, EAB I, III, IV, VI.
13. **Plagiochila drepanophylla** Sande Lacoste — Corticolous and ramicolous. WUS: 6953/L 6954/C. Lemurian species recorded the first time from the mainland by E. W. JONES (1980: 315–317) from our joint collection. As this locality is at 30 km distance from the above, the species seems to be widespread in the Usambaras, but not known elsewhere in continental Africa.
14. **Plagiochila effusa** Steph. — Corticolous. WUS: 6955/A. Widespread afromontane species, EAB I, VI.
15. **Plagiochila fusifera** Tayl. — Corticolous. WUS: 6950/H. Widespread in tropical Africa, EAB I, IV, VI.
16. **Plagiochila lastii** Mitt. — Corticolous. WUS: 6953/N. East African montane, EAB II, IV, VI.
17. **Plagiochila squamulosa** Mitt. var. **sinuosa** (Mitt.) Vanden Berghen — Ramicolous. WUS: 6951/M, P. East African montane, EAB I, III, VI.
18. **Plagiochila terebrans** Lindenb. ex Nees et Mont. — Corticolous. WUS: 6953/G, K, 6954/G; ULU: 6966/G. Widespread afromontane species, EAB I, VI.

Schistochilaceae

19. **Gottschea englerana** (Steph.) Grolle & Zijlstra — Corticolous. WUS: 6953/B, 6954/D, K. Crystalline block mountains of East Africa + Madagascar, see E. W. JONES 1976: 39 and EAB III, for synonymy see GROLLE & ZIJLSTRA (1984: 89).

Radulaceae

20. **Radula flaccida** Lindenb. et Gott. — Epiphyllous. EUS: 6947/BR. Widespread tropical Afro-American species, EAB I, GRADSTEIN, PÓCS, VÁNA 1984: 137.
21. **Radula holstiana** Steph. — On streambed rocks. EUS: 6947/AB. Widespread afromontane species, see map in OCHYRA, PÓCS 1982: 366.
22. **Radula recurvifolia** Steph. — On bark and on ramicolous bryophytes. WUS: 6951/Q, 5955/O. Widespread afromontane species, EAB I, IV, VI.
23. **Radula stenocalyx** Mont. — Epiphyllous. ULU: 6966/AJ, 6967/AH. Tropical Afro-American species, see map in GRADSTEIN, PÓCS, VÁNA 1984: 142.
24. **Rafula stipatiflora** Steph. — On shady rocks. *WUS: 6957/N. Afromontane species, EAB IV.

Porellaceae

25. **Porella hoehneltii** Steph. — Ramicolous. *ULU: 6967/O. Afromontane species new to the Ulugurus, EAB I, IV, V, VI.
26. **Porella subdentata** (Mitt.) E. W. Jones var. *subdentata* — Corticolous. *WUS: 6950/G. Widespread tropical African species, new to the Usambaras, EAB I, IV, VI.

Frullaniaceae

27. **Frullania apicalis** Mitt. — On bark, rocks and epiphyllous. EUS: 6947/BK, 6946/F p.p.; WUS: 6957/G; ULU: 6969/D. Widespread afromontane species, EAB III, IV.
28. **Frullania arecae** (Spreng.) Gott. — Corticolous. WUS: 6951/O. Widespread pantropical species, EAB I, II, VI.
29. **Frullania capensis** Gott. — On ericaceous bark. WUS: 6960/Q. Southeast African — Lemurian species, EAB IV.
30. **Frullania diptera** (Lehm. et Lindenb.) Gott. — Corticolous. *WUS: 6955/K. Tropical African, EAB I, III, uncommon.
31. **Frullania ericoides** (Nees) Mont. — Corticolous. WUS: 6951/N. Very widespread pantropical species.
32. **Frullania lindenberghii** Lehm. — Corticolous. EUS: 6946/F. SE African — Lemurian species, EAB III.
33. **Frullania obscurifolia** Mitt. — Corticolous. WUS: 6955/N. Tropical African species, EAB I, IV. ULU: 6972/K.
34. **Frullania serrata** Gott. — Corticolous. ULU: 6969/L. Widespread palaeotropical montane species EAB I, II, III, IV.

Lejeuneaceae

35. **Caudalejeunea lewallei** Vanden Berghen — Epiphyllous. *EUS: 6947/BV; ULU: 6966/AZ. East African montane, from Ethiopia to Burundi and to Zimbabwe, new for the Usambaras.
36. **Mastigolejeunea nigra** (Steph.) Steph. — Corticolous. EUS: 6946/K. Tropical African lowland species, from Guinea to the Zaire, sporadically in the Usambaras.
37. **Schniffneriolejeunea polycarpa** (Nees) Gradst. — Corticolous. WUS: 6955/X; ULU: 6972/E. Widespread tropical African species, EAB I, III.
38. **Marchesinia moelleriana** Pears. — On bark and on rocks, also epiphyllous. WUS: 6950/F, 6955/A, 6957/H, 6960/AA, 6372/DS. East African montane species, EAB I.
39. **Odontolejeunea tortuosa** (Lehm. et Lindenb.) Steph. — Epiphyllous. *EUS: 6946/AC; *WUS: 6947/BJ, 6374/BS; ULU: 6966/AS. Tropical African species, related to the tropical American *O. lunulata* (Web.) Schiffn., new to the Usambaras, EAB I, V.
40. **Ceratolejeunea calabariensis** Steph. — Epiphyllous and on bark. EUS: 6947/BU, 6948/D; *ULU: 6966/AQ. Tropical African, new to the Ulugurus, EAB I, IV.
41. **Leucolejeunea uncioloba** (Lindenb.) Evans. — On bark. WUS: 6955/Z. Afro-American species, in the African continent known only from the WUS: Mazumbai, Bumbuli-Soni and from the Southern Highlands of Tanzania: Mufindi, at 1500–1750 m alt., then in S-Africa. (Cf. E. W. JONES 1973: 547 and map in GRADSTEIN, PÓCS, VÁŇA 1984: 135).
42. **Cheilolejeunea (Cheilolejeunea) decursiva** (Sde-Lac.) Schust. — On bark. *WUS: 6955/U. Palaeotropic species, new to the Usambaras, EAB III, V (map on page 62).
43. **Cheilolejeunea (Cheilolejeunea) surrepens** (Mitt.) E. W. Jones — Epiphyllous. WUS: 6371/BA. East African — Lemurian species, EAB I, III, IV, under the synonym of *Cheilolejeunea silvestris* (Gott.) E. W. Jones var. *silvestris*.
44. **Cheilolejeunea (Euosmolejeunea) brachytoma** (Gott.) Schust. — On bark. EUS: 6946/A. Tropical African, EAB I, IV.
45. **Cheilolejeunea (Strepsilejeunea) brevifissa** (Gott.) Schust. — Epiphyllous. WUS: 6371/BF. East and South African montane species, EAB III, IV.
46. **Taxilejeunea conformis** (Mont.) Steph. — Epiphyllous. ULU: 6966/BB, 6967/AF. Widespread afromontane species, EAB I, III, IV.

47. *Pycnolejeunea angustiflora* Steph. — On *Philippia* (Ericaceae) bark. *WUS: 6960/DA. Very rare afromontane species described from Fernando-Poo, new for East Africa.
48. *Lejeunea* (*Hygrolejeunea*) *acuta* Mitt. — Epiphyllous. ULU: 6967/W. Afromontane, EAB I, III.
49. *Lejeunea alata* Mitt. Syn.: *Taxilejeunea mitricalyx* Eifrig. — Epiphyllous on leaves of Hymenophyllaceae. ULU: 6966/BD. 2nd record from continental Africa, palaeotropic, EAB III.
50. *Lejeunea caespitosa* Lindenb. ex G., L. et N. — On bark. WUS: 6950/J. Widespread tropical Afro-American species, EAB I, VI.
51. *Lejeunea flavovirens* J. Ångstr. — Corticolous. *WUS: 6953/H. New to the Usambaras, previously known only from Uganda: Muhawura volcano.
52. *Lejeunea* (*Microlejeunea*) *kamerunensis* (Steph.) Vanden Berghen — Epiphyllous. *WUS: 6372/DK. Afromontane species, new to the Usambaras, EAB I.
53. *Lejeunea* (*Hygrolejeunea*) *lyratiflora* (Steph.) Steph. 1892, Hedwigia 31: XIV. — Epiphyllous. ULU: 6966/AR. Afromontane species, EAB III.
54. *Lejeunea* (*Microlejeunea*) *ulicina* (Tayl.) Tayl. ex Gott. ssp. *ocellifera* (S. Arnell) Schust. — WUS: 6205/J, 6371/BD; ULU: 6966/AU. Tropical African subspecies, cf. GRADSTEIN, Pócs, Váňa 1984: 161, EAB I, II, III, IV, VI.
55. *Drepanolejeunea cultrella* (Mitt.) Steph. — Epiphyllous. EUS 6947/BN; ULU: 6966/AP, 6967/AA, 6968/D. Widespread afromontane species, EAB I, V.
56. *Drepanolejeunea physaeifolia* (Gott.) Steph. — Corticolous (especially on ericaceous bark) and on other bryophytes, also epiphyllous. *WUS: 6953/O, 6957/V, 6361/DU, 6960/Z; ULU: 6969/D. Montane species known from East Africa under the name of *D. friesii* Vand. Berg. and from Madagascar and the Mascarenes, EAB I, V. New for the Usambaras.
57. *Drepanolejeunea poessii* Grolle — Epiphyllous. ULU: 6966/AT, near the type locality. East African montane species, from South Kenya to Zimbabwe, EAB V.
58. *Drepanolejeunea trematodes* (Nees) Bischl. — Epiphyllous. ULU: 6968/K. East African — Lemurian montane species, EAB V.
59. *Leptolejeunea maculata* (Mitt.) Schiffn. — Epiphyllous. EUS: 6946/AB, 6947/BE; *WUS: 6371/AY; ULU: 6966/AF. EAB I, III.
60. *Prionolejeunea grata* (Gott.) Schiffn. — Epiphyllous on leaves of Hymenophyllaceae. ULU: 6966/BC. Widespread tropical African species, EAB I, III, the earlier synonym of *P. serrula* (Mitt.) Steph. fide Grolle 1978: 14.
61. *Cladolejeunea aberrans* (Steph.) Zwickel — Epiphyllous on *Trichomanes rigida* leaves. EUS: 6947/BB, not far from the type locality. The only record of this monotypic genus since its description from Amani. E. W. JONES (1974: 89) discusses this interesting taxon supplementing STEPHANI's and ZWICKEL's description in connection with a related group of species called by him, as *Lejeunea eckloniana* complex. He suspects its oil bodies to be simple, homogenous, in which case the *Lejeunea eckloniana* complex and *Cladolejeunea* would be congeneric. SCHUSTER (1980: 985) considers to be possible, that this complex should be separated, as a section of *Lejeunea*, JONES (l.c.) even counts with the possibility to place them in a separate genus. Both of them mention the name *Ciliolejeunea* S. Arnell as a possible name for the new section or of the genus including the whole complex.
The Author could examine materials from this taxon fresh enough to see its small, homogeneous, *Massula* type oil bodies. This fact seems to support JONES' anticipation of *Cladolejeunea aberrans* belonging to the "*Lejeunea eckloniana* complex". In this case the group at any level should bear the name of *Cladolejeunea* Zwickel 1933, not *Ciliolejeunea* S. Arnell 1953.
62. *Diplasiolejeunea cavifolia* (Steph.) Steph. — Epiphyllous. ULU: 6966/AH. Pantropical species, EAB I, III.

63. *Diplasiolejeunea cornuta* Steph. — Epiphyllous. EUS: 6947/BM. East African — Lemurian species, EAB I, III.
64. *Diplasiolejeunea kraussiana* (Lindenb.) Spr. — Epiphyllous. *EUS: 6947/BH. Southeast African species, new to the Usambaras, EAB III, V.
65. *Diplasiolejeunea symoensii* Vand. Bergh. — Epiphyllous. *EUS: 6946/AD; *WUS: 6371/BG, 6372/DN (coll. et det. E. W. JONES et T. Pócs). East African montane species, new to the Usambaras, EAB III.
66. *Diplasiolejeunea villaumei* Steph. — Epiphyllous. EUS: 6947/BO; WUS: 6371/AE, 6372/DJ. East African — Lemurian species previously known from East Africa under the name of *D. runssorensis* var. *australis* E. W. JONES, synonymized recently by P. TIXIER (1984: 21).
67. *Colura calyptrifolia* (Hook.) Dum. ssp. *calyptrifolia* — On *Philippia* (Ericaceae) bark. *WUS: 6960/E. *C. calyptrifolia* s. str. is an oceanic-temperate Afro-American (+ European) species and is very rare in tropical Africa, known only from Rwanda, Transvaal, Mauritius and new to the Usambaras (cf. GRADSTEIN, Pócs, VÁÑA 1984: 159), while the related *C. tenuicornis* Evans is more widespread.
68. *Colura digitalis* (Mitt.) Steph. — Epiphyllous. *EUS: 6946/AE, 6947/BG; WUS: 6371/BK; ULU: 6966/AX, 6967/AC, 6968/H; tropical African, EAB III.
69. *Colura dusenii* (Mitt.) Steph. — Epiphyllous. WUS: 6955/P; ULU: 6967/AD. Afromontane: from Cameroun to East Africa (cf. JOVET-AST 1976: 911).
70. *Cololejeunea africana* (Steph.) Schust. — Epiphyllous. *WUS: 6963/D, 6265/G, 6372/DR; ULU: 6966/AW. Tropical African species, EAB I, III.
71. *Cololejeunea apiculata* (E. W. Jones) Schuster — Epiphyllous. *ULU: 6966/AA. Afromontane species, new for East Africa, hitherto known only from its type locality on Mt. Cameroun at 1300 m alt.
72. *Cololejeunea appressa* (Evans) Ben. — Epiphyllous on *Trichomanes rigidum* leaves. *EUS: 6947/BD; ULU: 6966/AV. Pantropical representative of Subgenus *Taeniolejeunea* (Zwickel) Benedix, quite rare in Africa previously known only from Budongo Forest in Uganda, the Kasigau Hill in Kenya and from the Uluguru Mts. in Tanzania, EAB I, III.
73. *Cololejeunea bolombensis* (Steph.) Vanden Berghen — Epiphyllous. ULU: 6966/AL. Palaeotropical species, EAB I, III.
74. *Cololejeunea cardiocarpa* (Mont.) Evans — Epiphyllous. *EUS: 6946/AA; WUS: 6371/AZ, 6205/F; ULU: 6966/AO. Widespread pantropical species, EAB I, III, V.
75. *Cololejeunea crenatiflora* Steph. — Epiphyllous. EUS: 6947/BQ; ULU: 6966/AN. Tropical African species EAB III.
76. *Cololejeunea distalopapillata* (E. W. Jones) Schust. — Epiphyllous. EUS: 6947/BL; *WUS: 6963/H. East African montane, EAB I, III.
77. *Cololejeunea duvignaudii* E. W. Jones — Epiphyllous. ULU: 6966/AG. Tropical African, EAB I, III.
78. *Cololejeunea elegans* Steph. — Epiphyllous. *WUS: 6372/CS, coll. et det. by T. Pócs and E. W. JONES. New for the Usambaras, previously known only from Cameroun and from the Kasigau Hills in Kenya.
79. *Cololejeunea harrisii* Pócs — Epiphyllous. ULU: 6966/AB. East African montane species, EAB III.
80. *Cololejeunea hyalino-marginata* (Nees ex Mont.) Grolle — EUS: 6947/BF; ULU: 6966/AK, 6968/G. Widespread tropical African species previously known, as *C. leloutrei* (E. W. Jones) Schust., cf. EAB I, III.
81. *Cololejeunea malanjae* Steph. — Epiphyllous. *WUS: 6205/H; ULU: 6967/AE. SE African montane species, new from the Usambaras, EAB I, III.
82. *Cololejeunea minutissima* (Smith) Schiffn. ssp. *myriocarpa* (Nees et Mont.) Schuster; Syn.: *C. myriocarpa* (Nees et Mont.) Evans — Corticolous. *EUS: Amani, on planted

- trees near the Government Rest House, coll. T. Pócs et E. W. JONES, 6381/K. With squarrose, open leaves, the lobule not being closed to the lobe, typical to ssp. *myriocarpa*. A subspecies (or according to previous views, a species) hitherto known only from the Caribbean region of tropical America, new to Africa. With respect to the "weedy" character of this taxon and to its occurrence on a planted tree in a botanical garden, its occurrence might be also a recent introduction.
83. *Cololejeunea mocambiquensis* S. Arn. — Epiphyllous. *WUS: 6962/E. SE African — Lemurian species, EAB III.
 84. *Cololejeunea pusilla* Steph. var. *obtusifolia* E. W. Jones — Epiphyllous. *EUS: 6947/BS. Widespread tropical African species, EAB I, III, IV.
 85. *Cololejeunea tanzaniae* Pócs — Epiphyllous. *EUS: 6947/BT; WUS: 6371/AZ, 6372/DP; ULU: 6966/BD, 6967/AE, 6968/J. East African — Lemurian species, EAB V.
 86. *Cololejeunea usambarica* E. W. Jones — Epiphyllous. ULU: 6966/AM, 6967/AG. East African — Lemurian species, EAB III.
 87. *Aphanolejeunea exigua* Evans var. *africana* Pócs — Epiphyllous. *WUS: 6372/DT. East African variety of an Afro-American species (cf. Pócs 1983, 1984, Pócs, GRADSTEIN, VÁŇA 1984), new to the Usambaras.
 88. *Aphanolejeunea moramangae* P. Tixier — Epiphyllous. *WUS: 6371/BB. East African montane — Lemurian species (cf. TIXIER 1979, Pócs 1984), new for the Usambaras.

Metzgeriaceae

89. *Metzgeria agnewii* Kuwah. — Epiphyllous. *WUS: 6371/BJ. East African montane species, new to the Usambaras, EAB III, IV, VI, see map in OCHYRA, Pócs 1982: 369.
90. *Metzgeria leptoneura* Spruce — ULU: 6967/U; 6966/AY, 6967/AK. Oceanic subcosmopolite, EAB I, III.
91. *Metzgeria limbato-setosa* Steph. — *WUS: 6953/J. East African montane, EAB I, III, IV, VI.
92. *Metzgeria thomeensis* Steph. — Corticolous and epiphyllous. *EUS: 6947/AE; WUS 6372/DO; ULU: 6967/B, Q, R, T. Widespread tropical African species, EAB I, III, IV, VI

New taxa

93. *Cololejeunea amaniensis* Pócs, species nova (Plates I–II, Figs 1–17, 41)

Cololejeunea harrisii Pócs affinis, sed bene differt statura et cellulis minoribus foliis aureoviridibus, rhizoidis rubellis perianthiisque cordiformibus non auriculatis.

This species is related to *Cololejeunea harrisii* Pócs (1976: 357), but definitely distinct. Small, goldengreen plant creeping on filmy fern leaves. Shoot pseudo-dichotomously branching, up to 2 mm long, with leaves 0.6–0.8 mm broad. Stem diameter about 50 μ m. A conspicuous feature not known by any other African *Cololejeunea* species is the vine red colour of the rhizoids. They are slightly branching, connate at their base, forming a secondary rhizoid plate. Leaves distant to slightly imbricate, 0.4–0.6 mm long, 0.25–0.4 mm broad, widest near the middle, with broadly rounded apex. Marginal cells of lobi are elongated parallel to the margin, like by *C. harrisii*, but the outermost walls are incrassate. Other cell walls thin, without trigones or intermediate thickenings. Central lobe cells 20–25 μ m in diameter (up to 50 μ m by *C. harrisii*), isodiametric penta- or hexagonal. Basal and marginal cells are longer,

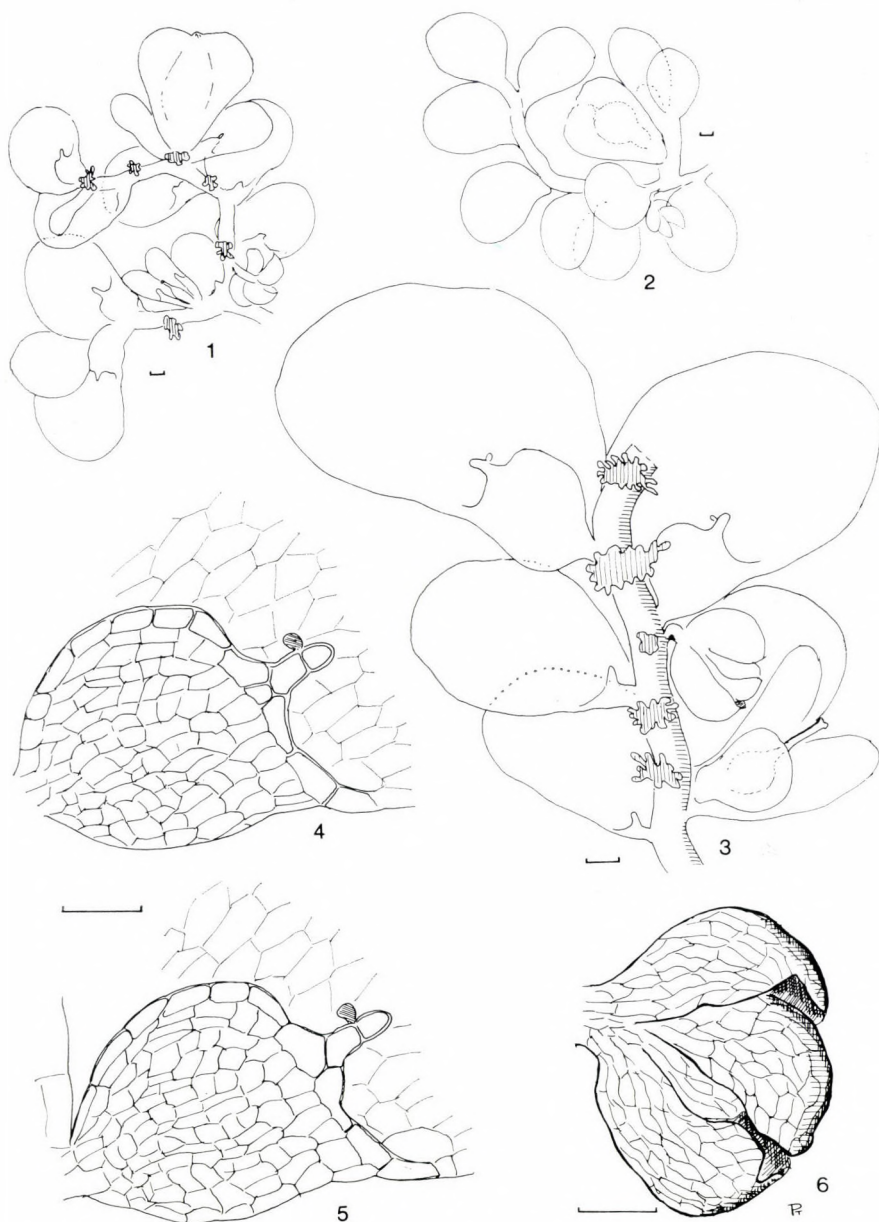


Plate I

Cololejeunea amaniensis Pócs sp. n. Figs 1–2. Shoot apices. Fig. 3. Part of shoot with perianths and male spikelets. Figs 4–5. Lobules. Fig. 6. Male spikelet. All drawn from the type. Scale bars represent always 50 μ m

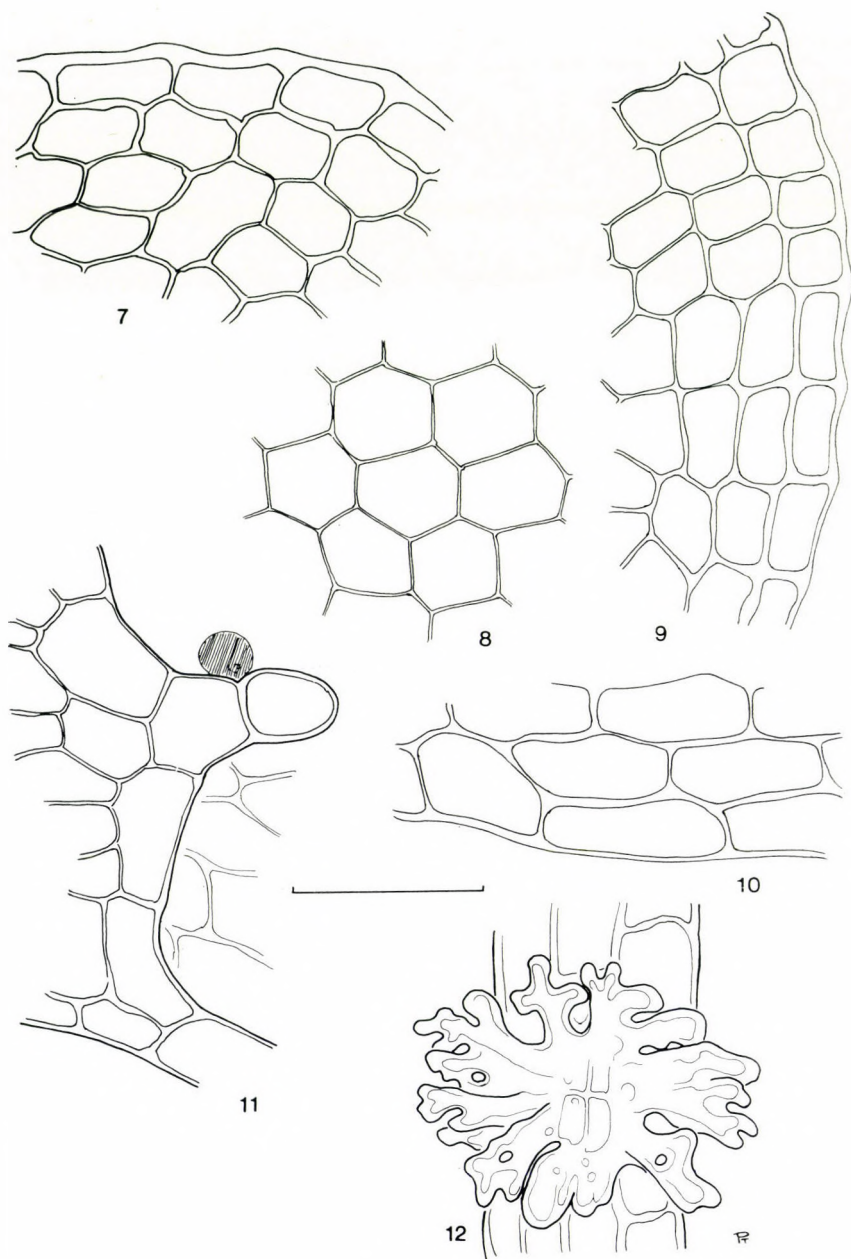


Plate II

Cololejeunea amaniensis Pócs sp. n. Fig. 7. Cells of the antical margin; Fig. 8 of lobe center; Fig. 9 of lobe apex; Fig. 10 of postical margin; Fig. 11 of lobule apex. Fig. 12. Rhizoid disc.
All drawn from the type

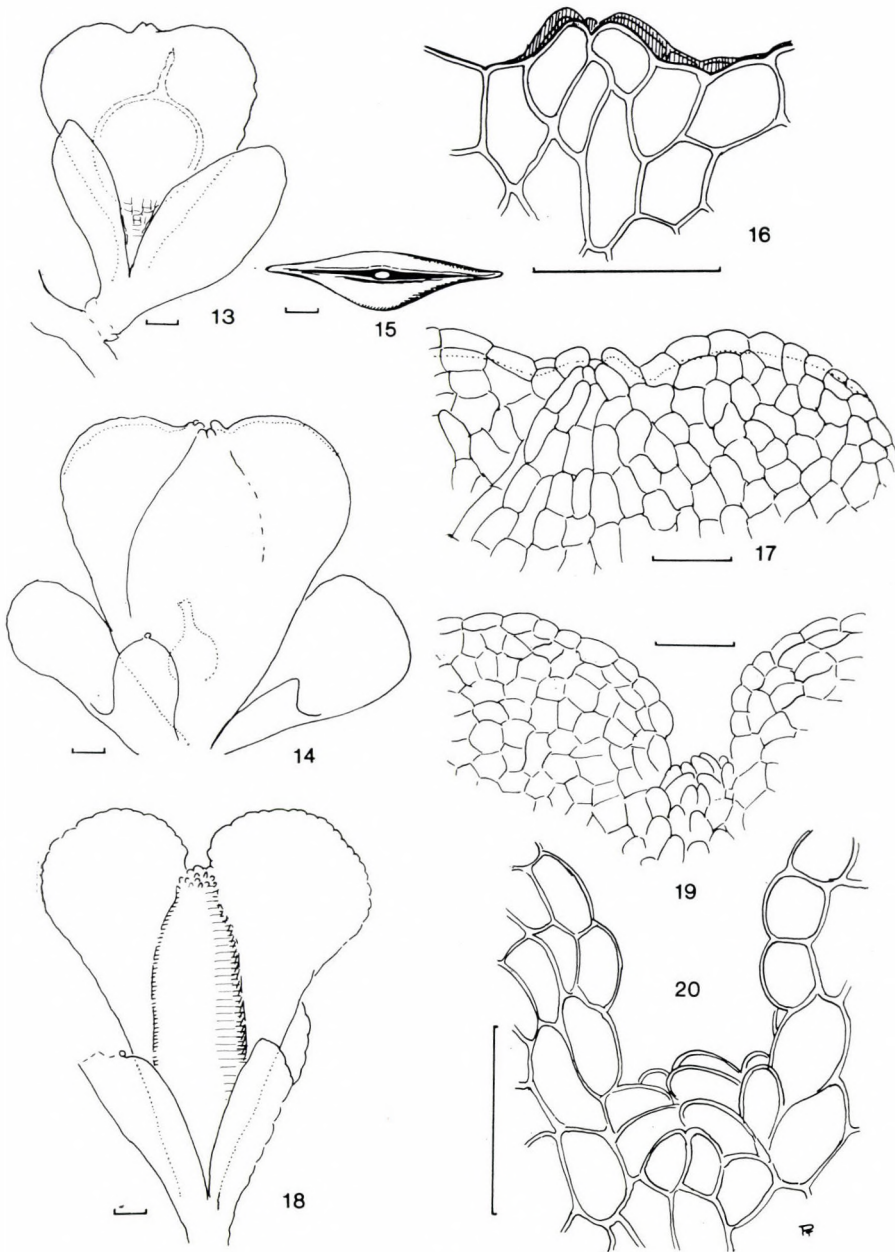


Plate III

Cololejeunea amaniensis Pócs sp. n. (Figs 13–17) and *C. harrisii* Pócs (Figs 18–20). Figs 13–14, Perianth, dorsal and ventral view. Fig. 15. Perianth in apical view. Figs 16–17. Perianth apex. Fig. 18. Perianth, ventral view. Figs 19–20. Perianth mouth. Figs 13–17. Are drawn from the type of *C. amaniensis*. Figs 18–20 from *C. harrisii* collected by DE SLOOVER in Rwanda, Rugege Forest, EGR

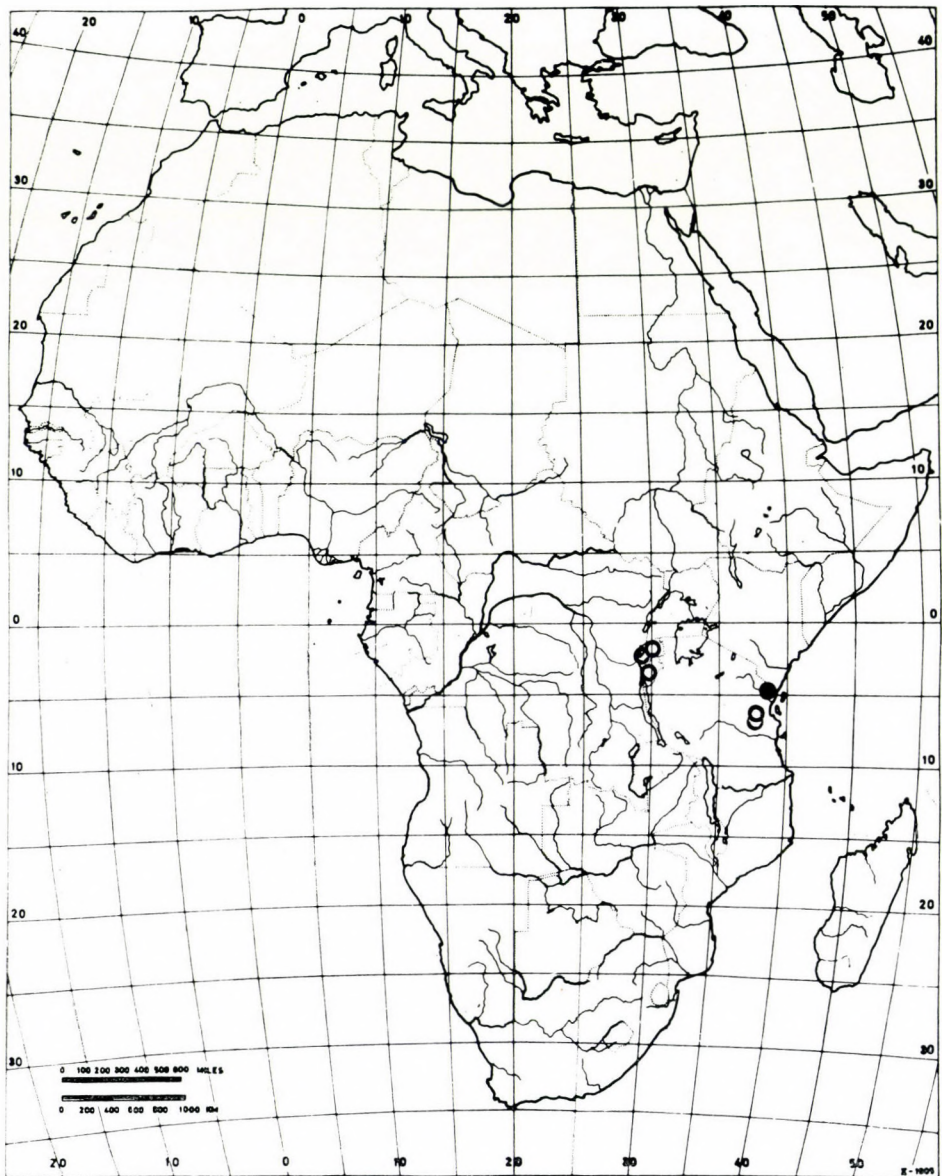


Fig. 41. Distribution of *Cololejeunea amaniensis* Pócs sp. n. (dot) and of *Cololejeunea harrisii* Pócs (open rings)

reaching $50\ \mu\text{m}$ in length, but only $10\text{--}15\text{--}(20)\ \mu\text{m}$ broad. Shape of the lobuli and the position of the hyaline papilla on the two-celled apical tooth is very similar to those of *C. harrisii*. Proximal tooth blunt, inconspicuous or lacking. Gemmae not seen.

Autoicous. Perianths develop on branches or on the main shoot, heart shaped, about $0.5 \times 0.5\ \text{mm}$ in size, compressed, their central part much less inflated than by *C. harrisii*

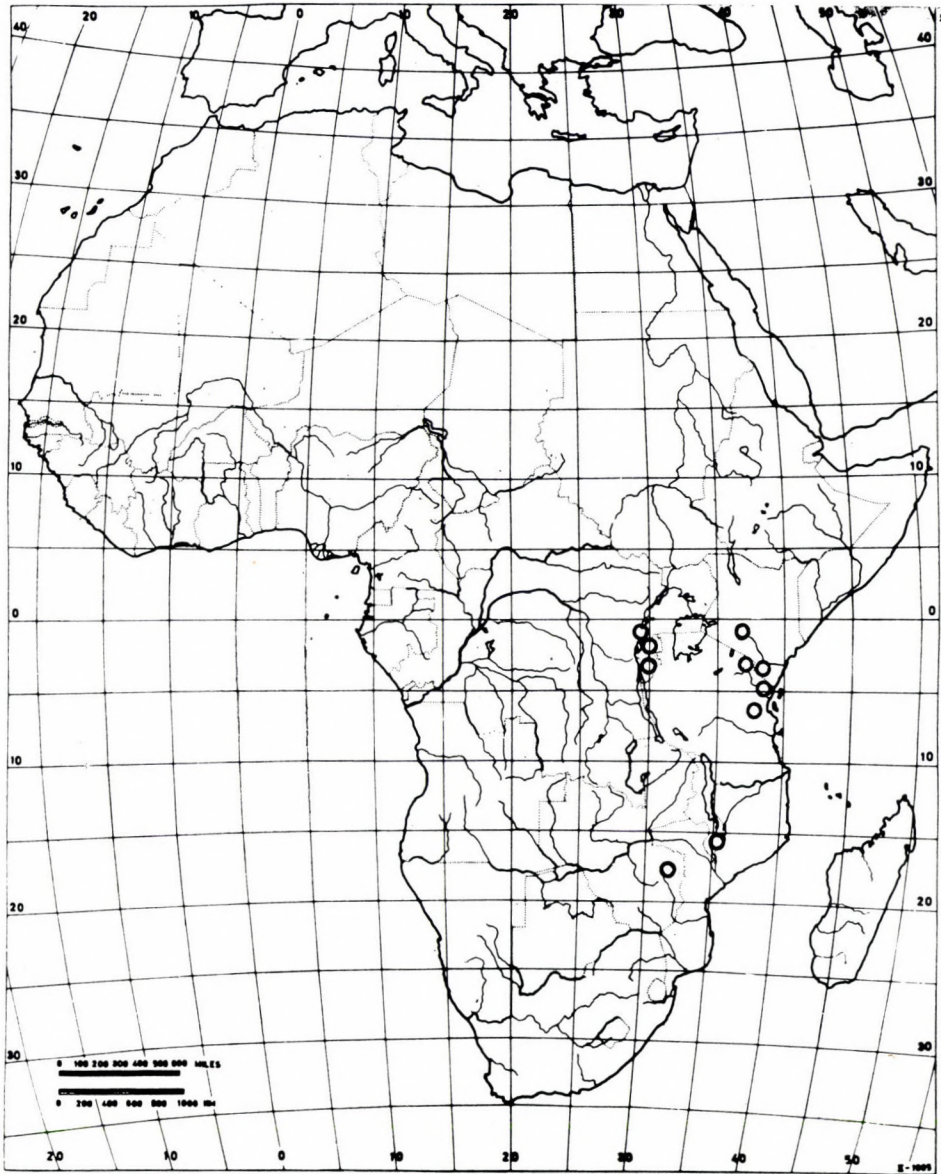


Fig. 42. Distribution of *Cololejeunea malanjeae* Steph.

and their wings are not auriculate and crenulate. Upper edges of the perianth halves are not connate, leaving the perianth open even in juvenile stage. Cells of the mouth are much less protruding, than those of *C. harrisii*. Female bracts broadly spatulate with smooth, entire margin, reaching half the length of the perianth. Male spikelets born on the main shoot, pendulous, consisting of 2-3 pairs of bracts.

Type: East Africa, Tanzania, Tanga Region, East Usambara Mts. Amani-Sigi Forest Reserve. Sigi headwaters area S of Amani, near Kwamkoro sawmills. Epiphyllous on *Trichomanes rigidum* leaves in submontane rain forest at 1000 m alt., accompanied by *Cladolejeunea aberrans* (Steph.) Zwickel. Collected by T. Pócs, No. 6947/BC, on 19. Feb. 1982. Holotype deposited in VBI, isotype in the microscopic slide collection.

Cololejeunea amaniensis and *C. harrisii* seem to form a natural group of African species, maybe at the section level. The affinities to other species are discussed by Pócs (1976, l.c.)

94. *Cololejeunea tanneri* Pócs, *species nova* (Plates IV–VI, Figs 21–40, 42, 43)

Cololejeuneae malanjae Steph. *similis, sed differt cellulis lobi distincte papillosis, papilla hyalina basi dentis apicalis disposita, atque carinis perianthii et bracteis femininis mamillatis crenulatis.*

Epiphyllous, it develops in vivid green patches of 3–5 mm in diameter. Stems of 2–4 mm length, 50 μ m in diameter, irregularly branching, shoot with leaves 0.6–0.9 mm broad, slightly appressed to the substrate. Rhizoids form secondary plates, colourless. Leaves contiguous or distant, 0.4–0.6 \times 0.16–0.27 mm in size, with often strongly, irregularly dentate margin. Marginal teeth uni- or pluricellular, broad triangular. Lobule inflated, unidentate with apical teeth of 2 cells long. Hyaline papilla at the inner base of the apical tooth, in central position. Lobe and lobule cells more or less isodiametric, in the centre penta- or sex-angular, 10–15 μ m, at the margin and apex more quadrangular, 8–12 μ m in diameter. Each lobe cell tipped with a large hemisphaeric or conical papilla on the dorsal side of the lobe, except its lowermost part. Cell walls evenly thickened at the lobe margin, with triangular thickenings in the centre and without thickening at the base of the lobe and in the lobule. Discoid gemmae 20 celled, develop on the ventral lobe surface, up to 80 \times 60 μ m size.

Autoicous, the perianths and male bracts born on branches. Perianth obovate, 5 keeled, 0.5 \times 0.35 mm. Carinae with conically protruding cells. No beak. Female bracts with crenulate margin and keel due to the conical mamillate cells, equalling the perianth in length. Male bracts similar to leaves, separate spikelets not being observed.

The new species is dedicated to Lucy and John TANNER, who donated the Mazumbai Forest Reserve and Research Station to the University of Dar es Salaam, for the benefit of science and of the Tanzanian people.

Type: East Africa, Tanzania, Tanga Region, West Usambara Mts. Mazumbai University Forest Reserve, near its S end, in submontane rain forest at 1500 m altitude, in a damp gully. Epiphyllous on *Marattia fraxinea* leaflets, associated with *Cololejeunea mocambiquensis* S. Arn. Coll. T. Pócs and A. BORHIDI 6963/F. Holotypus deposited in VBI, isotypes in DSM, G, UPS. Paratype: 6962/F, on filmy fern leaves (microslide in VBI).

Cololejeunea tanneri obviously belongs to a group of mostly African *Cololejeunea* species discussed by E. W. JONES (1953), as “*Cololejeunea* and *Leptocolea* with dentate leaves” where he enumerated *C. grossidens* Steph., *C. malanjae* Steph., *Leptocolea dentata* E. W. Jones from Africa, *C. pentagona* (Mitt.) E. W. Jones from Samoa and *C. decliviloba* (Steph.) E. W.

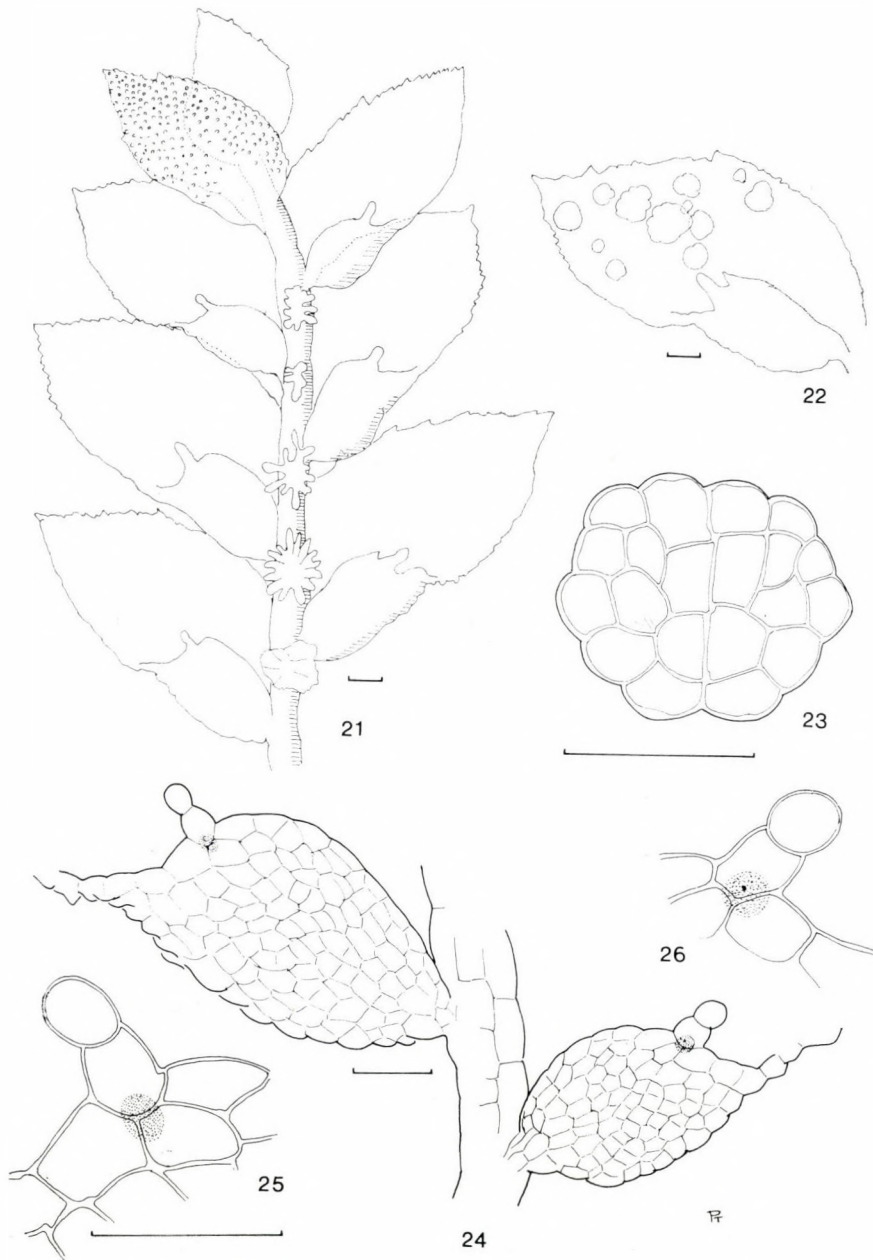


Plate IV

Cololejeunea tanneri Pócs sp. n. Fig. 21. Ventral view of a shoot apex, showing the dorsal side of a lobe. Fig. 22. Leaf with dis-coid gemmae on the ventral side of lobe. Fig. 23. Gemma. Fig. 24. Part of shoot showing two lobules. Figs 25-26. Apical tooth with hyaline papilla. All drawn from the type

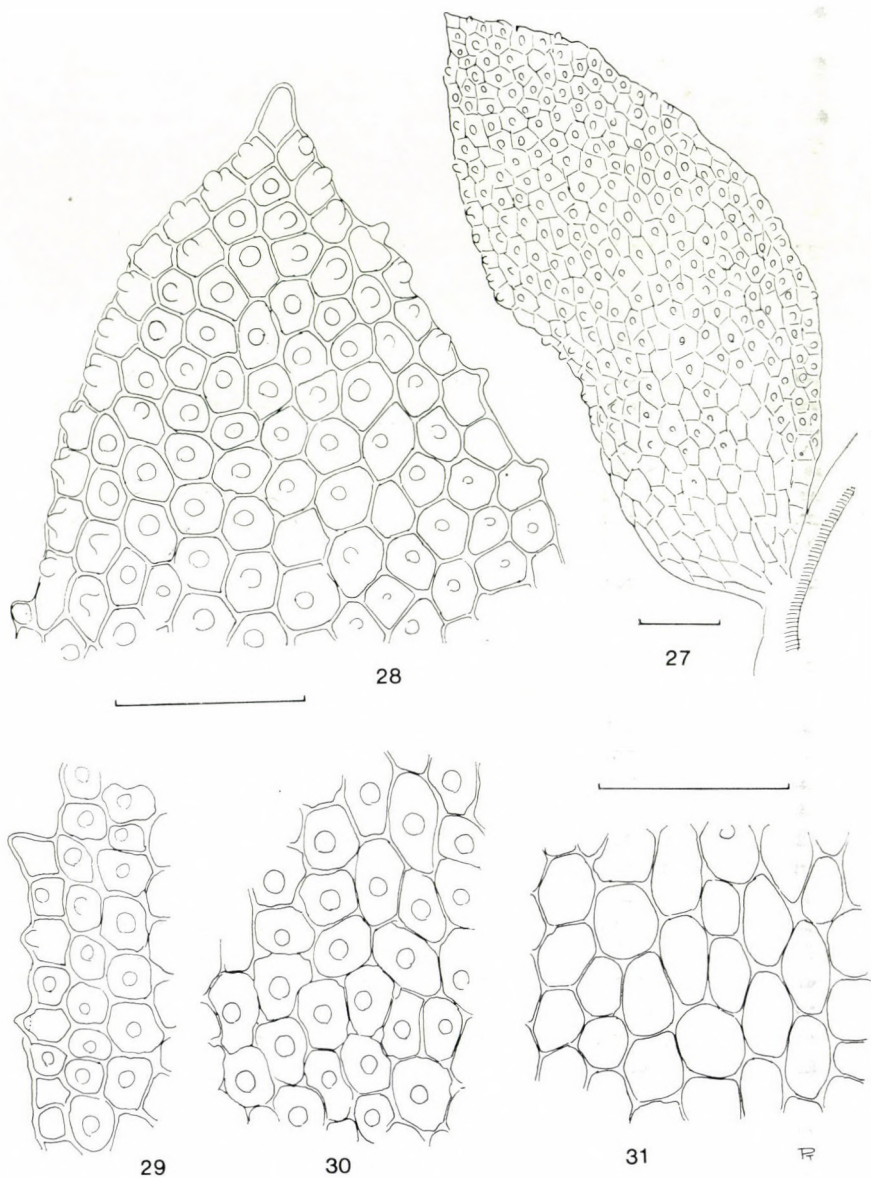


Plate V

Cololejeunea tanneri Pócs sp. n. Fig. 27. Leaf seen from its dorsal side. Fig. 28. Cells from the dorsal side of lobe apex; Fig. 29 of lobe margin; Fig. 30 of lobe center; Fig. 31 of lobe base. All drawn from the type

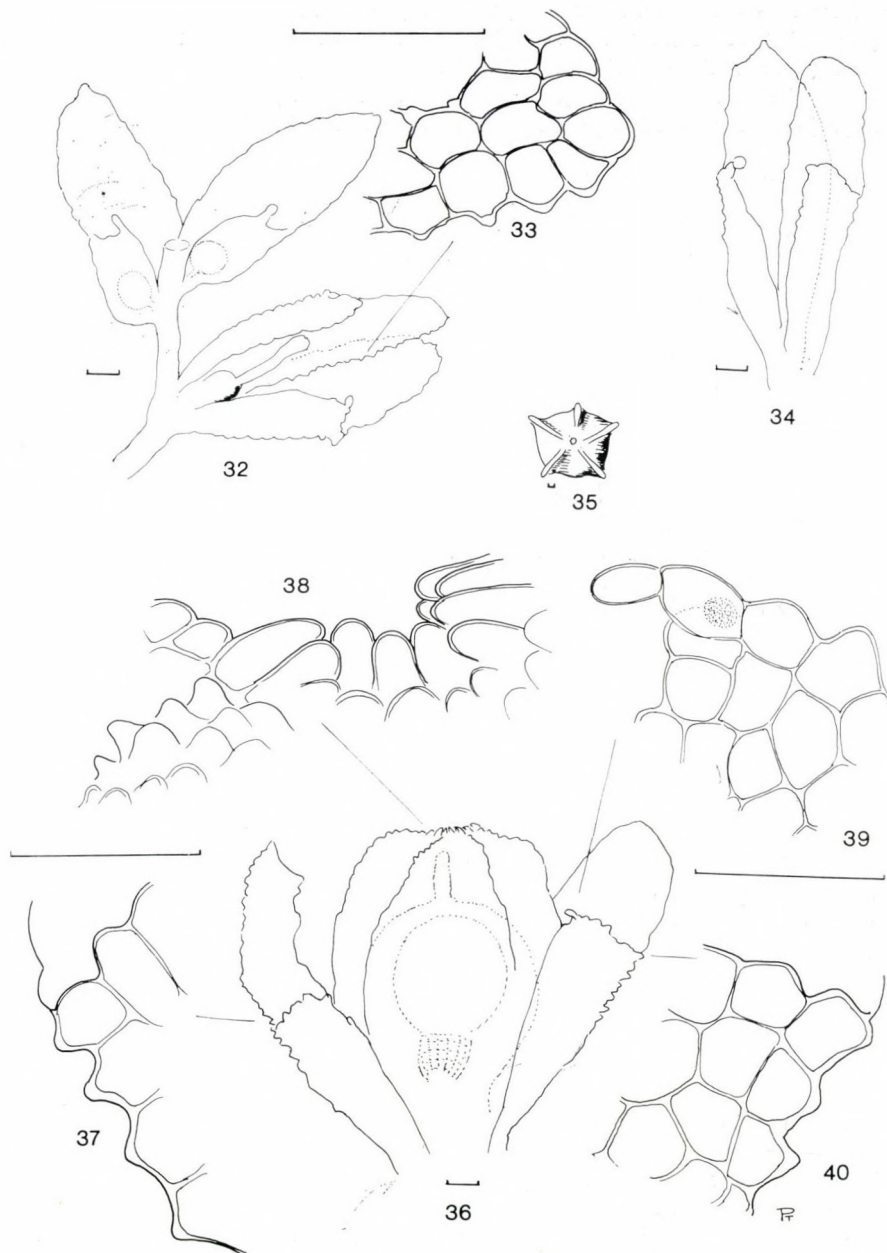


Plate VI

Cololejeunea tanneri Pócs sp. n. Fig. 32. Part of shoot with female and male bracts. Fig. 33. Apex of a female bract. Fig. 34. Female bracts. Fig. 35. Perianth, apical view. Fig. 36. Perianth with bracts, dorsal view. Figs 37 and 40. Keel with female bracts. Fig. 38. Perianth mouth. Fig. 39. Female bract lobule with tooth and hyaline papilla. All drawn from the type

Jones from New Caledonia. SCHUSTER (1963: 175) created for this or at least for a part of this group his Sectio *Dentatae*. *C. malanjae* Steph. and *C. grossidens* Steph. were later synonymized (VANDEN BERGHE 1978: 452) and further new species added to the same group: *Cololejeunea borhidiana* Pócs (1980), *C. calcarata* E. W. Jones (in JONES, HARRINGTON 1983). JONES (l.c.) considers the Madagascan *C. plagiophiliana* P. Tixier also closely related. An other

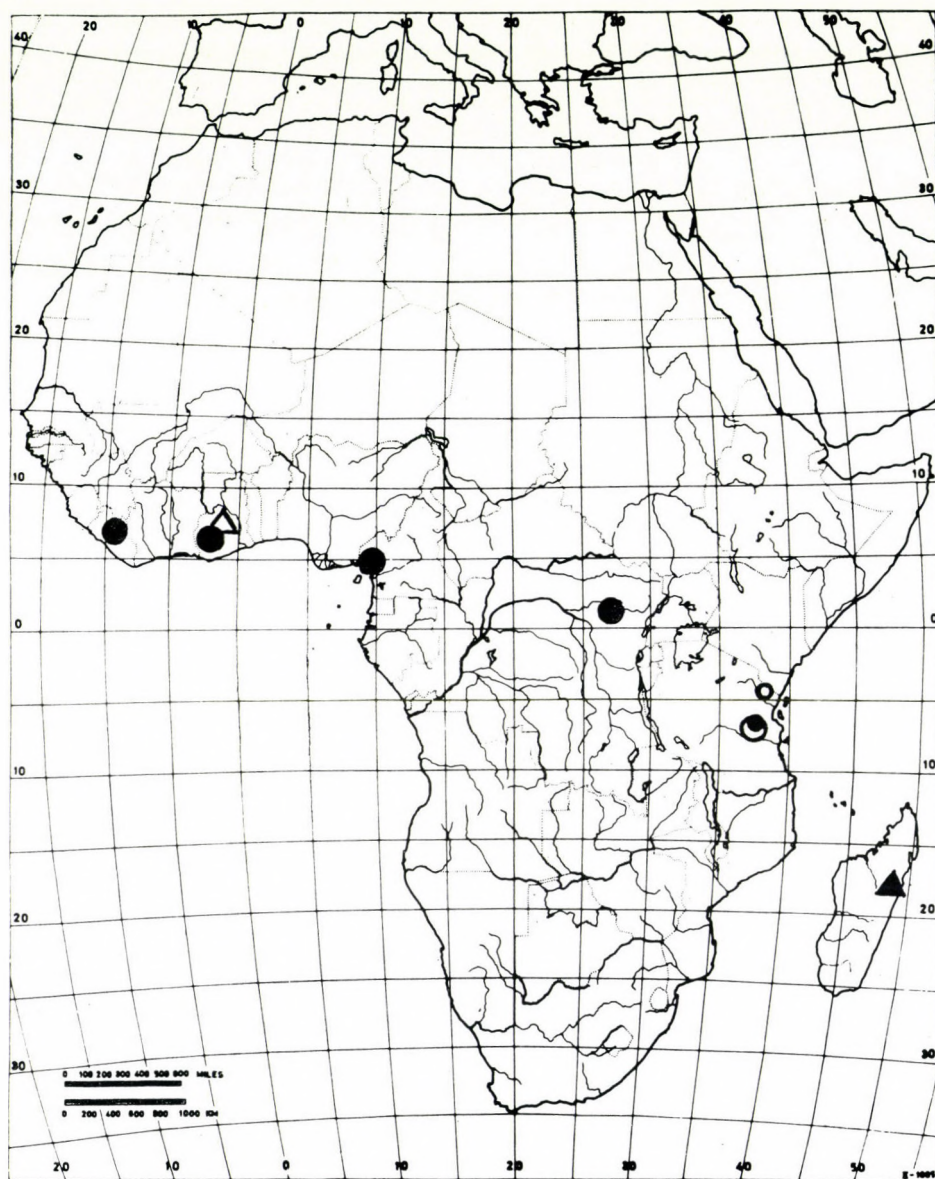


Fig. 43. Distribution of *Cololejeunea tanneri* Pócs (small ring), of *C. borhidiana* Pócs (large ring), of *C. grolleana* Pócs (small dot), of *C. dentata* (E. W. Jones) Schust. (large dot), of *C. calcarata* E. W. Jones (open triangle), and of *C. plagiophiliana* P. Tix. (full triangle)

species, *Cololejeunea grolleana* Pócs (1980) with large, sphaeric papillae on the lobe surface and with very broad, serrulate leaves, seemed to be isolated. Now, in the knowledge of the above new species, which has intermediate position in some aspects between *C. grolleana* and members of the Sectio *Dentatae*, it seems to be clear to count *C. grolleana* too to the same group, with dentate-serrate and acute lobe, with gemmae on its ventral surface. In addition to *C. decliviloba* Steph. a set of New Caledonian species described by TIXIER (1979) also should belong here (*C. pseudoserrata* P. Tix., *C. mackeeana* P. Tix., *C. huerlimannii* P. Tix. and *C. ningwana* P. Tix.). The perianth in *Dentatae* can vary from cornute or 5 carinate to smooth, compressed, the proximal lobule tooth from long, falcate to nil, cell walls from smooth through conically protuberant to strongly papillate. The Sectio represents a palaeotropic group of species, centered in the old, Gondwanalandic areas of New Caledonia and of East Africa, where the widest distributed species, *C. malanjae* lives and where strong speciation took place, and even more locally distributed species can be expected (cf. figs 42, 43).

Phytogeographical conclusions

At the present level of knowledge on the liverwort flora of the Usambara Mountains it should be premature to carry out a detailed analysis on the floristic composition. Anyway, compared to other parts of East Africa, the lowland and submontane rain forests of East Usambara Mountains bear a large number of tropical African lowland species mostly distributed in West Africa like *Frullania diptera*, *Mastigolejeunea nigra*, *Cheilolejeunea brachytoma*, *Colura digitalis* and *Cololejeunea hyalino-marginata*. On the other hand, afro-montane or East African montane species occur mostly in the montane rain forests at higher altitudes of the West Usambaras, like *Plagiochila effusa*, *P. lastii*, *P. squamulosa*, *P. terebrans*, *Radula recurvifolia* and *stipatiflora*, *Gottschea angustiflora* and *Lejeunea kamerunensis*, *Drepanolejeunea cultrella*, *Diplasiolejeunea symoensii*, *Colura dusenii*, *Cololejeunea malanjae*, *Metzgeria agnewii* and *M. limbato-setosa*.

The most peculiar group is formed by species, which are widespread in Madagascar and in the Mascarene Islands while on the mainland are restricted mostly to the old crystalline blocks of East Africa. Very few of them occur on the much younger East African volcanoes. The distribution pattern of these East African — Lemurian species is discussed in details by BIZOT and Pócs (1974: 393) and by Pócs (1975: 126, 1982: 303–305). Their number is maybe the highest in the Usambara and Uluguru Mountains in continental Africa. From the present enumeration the following species belong to this group: *Bazzania borbonica* (from the Ulugurus to Transvaal), *Plagiochila cambuena* and *P. drepanophylla* (the latter only in the Usambara), *Gottschea englerana*, *Frullania capensis*, *F. lindenberghii*, *Cheilolejeunea surrepens*, *Lejeunea flavovirens*, *Drepanolejeunea physaefolia*, *D. trematodes*, *Diplasiolejeunea cornuta* and *D. villaumei*, *Cololejeunea mocambiquensis*, *C. tanzaniae*, *C. usambarica* and *Aphanolejeunea moramangae*.

Finally we can consider to be endemic in the Usambaras according to our present knowledge *Plagiochila angustitexta*, *Cladolejeunea aberrans*, *Cololejeunea amaniensis* and *Cololejeunea tanneri*.

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EAST AFRICAN BRYOPHYTES, VIII
THE MUSCI OF THE USAMBARA RAIN FOREST PROJECT
EXPEDITION, 1982*

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Forty-nine mosses from the Usambara and Uluguru Mts. are reported. *Leucoloma sinuosulum*, *Syrrhopodon asper* and *Forsstroemia producta* are new to the Usambaras, *Dicranella heteromalla* and *Orthodontium lineare* to Tanzania, finally *Leucophanes angustifolium* and *Papillaria flexicaulis* to the flora of the African continent. The distribution of southern temperate *Dicranoloma billarderi* is discussed in details and accompanied by a map. Characteristic habitats of the Usambara Mts. are pictured.

Keywords: Musci, Usambara, Uluguru, Tanzania, Africa

Introduction

This paper contains records on the greater part of mosses collected during the same preparatory expedition of Tanzanian-Swedish-Hungarian Usambara Rain Forest Project in 1982, as the previous paper (East African Bryophytes VII, Pócs 1985), in which the liverworts were enumerated. The collector was T. Pócs, a member of the expedition.

Abbreviations used in the enumeration:

EUS: East Usambara Mountains

WUS: West Usambara Mountains

ULU: Uluguru Mountains

det. RO: identified by R. OCHYRA

det. SO: identified by S. ORBÁN

det. P: identified by T. PÓCS

The localities are indicated by the same numbers, as in the previous paper (Pócs 1985), therefore not listed again. After the localities general information on the distribution of species and reference to records in the previous issues of the East African Bryophytes series ("EAB") are given. The collected specimens were deposited in DSM, UPS and VBI herbaria.

ACKNOWLEDGEMENTS

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* Usambara Rain Forest Project Publications, No. 2.

LIST OF SPECIES COLLECTED

Dicranaceae

1. *Dicranella heteromalla* (Hedw.) B. S. G. — WUS: 6960/V, det. RO. A very widespread circumboreal species known also from Macaronesia. South of the Holarctic it is known only from the Andes and in East Africa. HERZOG (1916) reports the species from Bolivia and states that the Colombian *D. crassinervis* (Hampe) Jaeg. is conspecific with it. In tropical Africa *D. heteromalla* was collected only once in Kenya (DIXON 1938, SAPP and PIOVANO 1947), where it was found at 2900 m altitude. The present Tanzanian specimens were collected in excellent fruiting conditions.
2. *Campylopus chrismarii* (C. Müll.) Mitt. — WUS: 6956/B, det. RO. Highly variable and widely distributed Afro-American montane species. Its taxonomy and variability in Africa was recently discussed by DE SLOOVER (1982). At the same time FRAHM (1982) found the African *C. stramineus* Mitt., and the Mascarenian *C. commersonii* Besch. and *C. boryanus* Besch. to be conspecific with the Central and South American *C. chrismarii* thus extending the presently known range of this species from America to West and East Africa including Madagascar and Réunion Islands. This way the number of common Afro-American moss species increased and show the same regularities as observed by liverworts (GRADSTEIN et al. 1984). EAB I, III, IV, VI.
3. *Dicranoloma billardieri* (Brid. ex anon.) Par. — WUS: 6960/A, det. RO. A southern pan-temperate species. It occurs in the temperate areas of southern South America, Australasia and South Africa. In South America it is distributed on Juan Fernandez Islands (ROBINSON 1975) and along the west coast of the continent (DUSEN 1905, CARDOT and BROTHERUS 1923, SEKI 1974), in Tierra del Fuego (BARTRAM and ROIVAINEN 1937) and on the Falkland Islands (CARDOT 1908). The species is widespread on the sub-antarctic islands of the Indian Ocean sector (VAN ZANTEN 1971) as well as on Macquerie Island (SEPPELT 1981), on Campbell Island (VITT 1979) and in New Zealand (SAINSBURY 1955). In Australia *D. billardieri* occurs in the southeastern part of the continent from Queensland to Tasmania and South Australia (CATCHESIDE 1980). It occurs also in the Eastern Highlands of New Guinea (a specimen collected by B. O. VAN ZANTEN at 3500 m altitude, No. 68739, EGR). Recently ROSARIO and VAN ZANTEN (1982) reported *D. billardieri* from Luzon Island and TAN and KOPONEN (1983) after revision of different southeastern Asian species of *Dicranoloma* found them to be conspecific also with this widely distributed species. Thus, at present *D. billardieri* is known to occur on Mt. Kinabalu, Borneo, from where it was described as *D. subenerve* Broth., in Gunong Tahan on Malay Peninsula from where it was described as *D. brevicapsulare* Dix. One additional record is known also from Mindanao Island in the Philippines from where it was reported as *D. philippinense* Bartr. and an other from Papua New Guinea (Mt. Dayman, Milne Bay Distr.). The species is known also from New Caledonia (TIXIER 1979, MILLER, WHITTIER and WHITTIER 1978). In Africa it is frequent in the southernmost part of Cape (MAGILL 1981), occurs in Transvaal and penetrates as high as 3000 m altitude in the montane forests, ericaceous heaths and elfin woodlands in East Africa (BIZOT and PÓCS 1974: 420, 1980: 249). It is widely distributed also on the East African islands (CROSBY et al. 1983). Reports on the occurrence of this moss from the high elevation of the Peruvian Andes (MITTEN 1869, P. and E. HEGEWALD 1975) correspond well with its localities in the high mountains of East Africa and Indomalaysia. The species follows the pathways of other Gondwanalandic temperate elements penetrating northwards into the tropical mountains (cf. SCHUSTER 1983, GRADSTEIN et al. 1984). Annotation by RO and P (see Fig. 1).

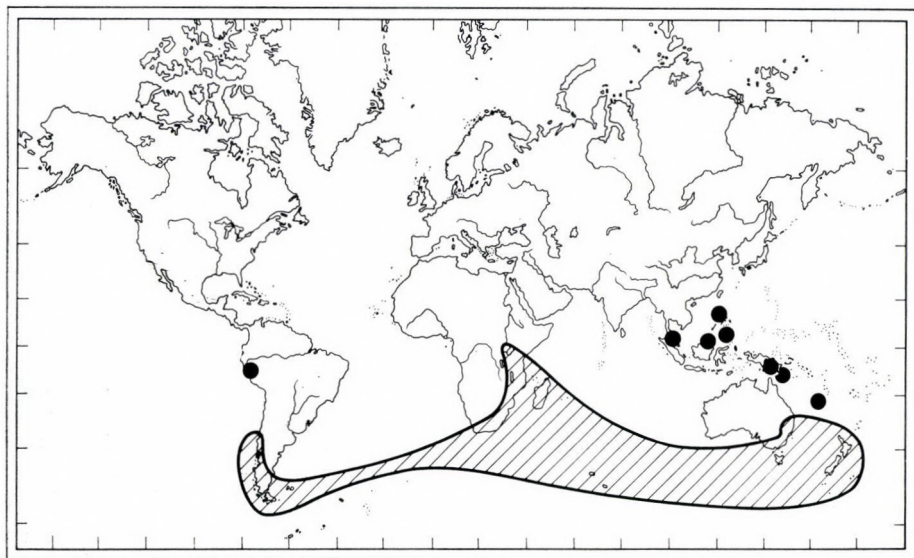


Fig. 1. World distribution of *Dicranoloma billarderi*, a southern pan-temperate species. Map made by R. OCHYRA and T. PÓCS

4. *Leucoloma aspericuspis* P. Varde — WUS: 6955/C, 6960/S, det. P. East African montane species, map in EAB II. (+ Mt. Kilimanjaro, cf. Bizor et al. 1979: 153).
5. *Leucoloma holstii* Broth. — EUS: 6947/X, 6948/A, det. RO; WUS: 6959/C, det. P. Widespread East African montane species originally described from the Usambara Mts. EAB II, III, IV, VI.
6. *Leucoloma sinuosulum* C. Müll. ex Besch. — WUS: 6954/B, 6957/E, 6959/B, det. P. Examining the specimens recorded earlier from East Africa as *L. brotheri* Ren. (Bizor and Pócs 1974: 420), they all proved to be *L. sinuosulum*, characterized by narrower hyaline margin (only 8–12 cells, while 15–25 cells broad by *L. brotheri*) and by the stellate, multifid dorsal papillae (bifid by *L. brotheri*). Therefore *L. brotheri* is to be cancelled from the East African flora, and the two records from Uluguru Mts. should be added to the known distribution of *L. sinuosulum*, which is a Lemurian species, previously known from East Africa only from Uluguru and Nguru Mts. EAB I, III. Annotation by P.
7. *Leucoloma volkensii* Broth. — ULU: 6967/S, det. P. East African montane species, EAB I, III.
8. *Leucobryum isleanum* Besch. var. *molle* (C. Müll.) Card — EUS: 6947/XB, 6948/E, det. RO. Widespread East African — Lemurian species, EAB I, IV, V.

Calymeraceae

9. *Leucophanes angustifolium* Ren. et Card. — EUS: 6947/AA, det. P. New for continental Africa, previously known only from Réunion Island; Lemurian species. In the Usambaras occurs on tree buttresses.
10. *Leucophanes hildebrandtii* C. Müll. — ULU: 6966/J, det. P. Lemurian species, EAB I, III.
11. *Syrhobodon gaudichaudii* Mont. — WUS: 6955/S, 6956/C, det. SO. Widespread tropical Afro-American species (ORBÁN 1981: 175).

12. *Syrrhopodon asper* Mitt. — EUS: 6947/W; WUS: 6954/L, 6955/R, 6960/G; ULU: 6966/E det. SO. Afromontane species, new for the Usambaras (ORBÁN l.c.).
13. *Syrrhopodon stuhlmannii* Broth. — ULU: 6967/Y, det. SO. Endemic of the Uluguru and Nguru Mts. (map in ORBÁN 1977: 175).

Pottiaceae

14. *Barbula indica* (Hook.) Spreng. — ULU: 6970/A, det. RO. Widespread pantropical species with sporadic occurrence also in the warm temperate zones. In Africa: East and South and on the offshore islands, EAB I, III.

Bryaceae

15. *Orthodontium lineare* Schwaegr. — WUS: 6955/W, det. P. New to Tanzania. The species is widespread in South Africa, and recently has been introduced in Western Europe from where it extends its range eastwards (MEIJER 1952, OCHYRA 1982). MEIJER in his world-wide revision (1952) accepted a narrow concept of species and recognized three subspecies within *O. lineare*. Another southern species *O. australe* Hook. f. and Wils, including two subspecies MEIJER considered to be species of its own. However, VAN ZANTEN (1971) showed convincingly that there are no reasons to keep these two species separately. The same is true in the case of the infraspecific taxa of *O. lineare* and in consequence OCHYRA (1982) synonymized all these subspecies with the highly polymorphic *O. lineare*. Annotation by RO.



Fig. 2. East Usambara Mts. Corticolous community with *Mastigolejeunea nigra*, *Cheilolejeunea brachytoma*, *Frullania apicalis* and *F. lindenbergii* in the submontane rain forest near Amani "Forest Houses", locality 6946. Photos made by T. Pócs

16. *Bryum alpinum* Huds. ex With. s. lato vel sp. aff. — WUS: 6964/A, det. P. A specimen living among dry conditions and showing characters intermediate between *B. alpinum* and *B. muehlenbeckii* B. S. G. Cosmopolite, montane in tropical Africa, EAB I, III.
17. *Bryum huillense* Welw. et Dub. — EUS: 6946/P; ULU: 6967/P, 6972/A, det. P. Widespread pantropical species, EAB III.
18. *Bryum keniae* C. Müll. — EUS: 6948/G; ULU: 6969/G, det. P. Afromontane species, EAB I, III, IV, VI.

Mniaceae

19. *Plagiomnium rhynchophorum* (Hook.) T. Kop. var. *rhynchophorum* — ULU: 6971/D, det. P. Pantropical species, widespread in the tropical African mountains, new for the Ulugurus. EAB IV.

Rhizogoniaceae

20. *Rhizogonium spiniforme* (Hedw.) Bruch. — EUS: 6947/Y, det. P. Widespread pantropical species, EAB I, IV, V, VI.

Bartramiaceae

21. *Philonotis hastata* (Duby) Wijk et Marg. — ULU: 6970/F, det. RO. Very widespread pantropical species, EAB I, IV, V, VI.

Orthotrichaceae

22. *Macromitrium levatum* Mitt. — WUS: 6960/T; ULU: 6968/K, 6969/J, K, S, det. RO. Widespread afromontane species occurring in the whole tropical Africa from Guinea to Madagascar. EAB I, IV, V.
23. *Macrocoma tenue* (Hook. et Grev.) Vitt — ULU: 6972/G, det. P. Tropical Afro-American species, EAB I, VI.



Fig. 3. West Usambara Mts. The Mazumbai University Forest Reserve on the E slopes of Sagara ridge, 1850–1400 m, covered by submontane and montane rain forests. Localities 6959–73

Racopilaceae

24. **Racopilum capense** C. Müll. — ULU: 6972/D, det. RO. Widespread in tropical and in South Africa, EAB IV, VI.

Hedwigiaceae

25. **Rhacocarpus purpurascens** (Brid.) Par. — ULU: 6969/H, det. P. Southern temperate with distribution pattern similar to that of *Dicranoloma billarderi* (see page 136), but with an extension northwards through the Andes up to Mexico. In Africa it occurs in the Cape area and in the high mountains of East Africa, then at the offshore islands. EAB I, map on page 396.

Cryphaeaceae

26. **Forsstroemia producta** (Horsch.) Par. — WUS: 6951/L, det. RO. A widespread element of the dry forests of South and East African mountains, new to the Usambaras, EAB IV, V, VI with map on page 377.

Rutenbergiaceae

27. **Neorutenbergia usagarae** (Dix.) Biz. et Pócs — WUS: 6954/A, 6960/B. East African montane species restricted to the old crystalline blocks, EAB V. (Map: Pócs 1975: 127.)

Trachypodiaceae

28. **Trachypus bicolor** Reinw. et Hornsch. var. **viridulus** (Mitt.) Zant. — WUS: 6957/J, det. RO. Pantropical, montane in tropical Africa, EAB I, V.

Pterobryaceae

29. **Renauldia lycopodioides** Biz. ex Pócs — WUS: 6960/F, det. P. Collected at its type locality 12 years after its discovery. Uncommon, endemic of West Usambara Mts.
30. **Pterobryon julaceum** Broth. — WUS: 6957/F, det. RO. It grows on half shady granitic rocks accompanied by *Drepanolejeunea physaefolia*, *Frullania apicalis*, *Marchesinia moelleriana*, *Plagiochila divergens*, *Radula stipatiflora*, *Catagonium nitens*, *Leucoloma sinuiculum* and *Trachypus bicolor* var. *viridulus*. *Pterobryon julaceum* is undoubtedly one of the most interesting record of mosses in this area. It is an endemic taxon of the Usambara Mts. and actually is known only from two collections: the type material collected by HOLST in Bumba (BROTHERUS 1894), and a second collection made by GREENWAY from Usambara (without precise locality, cited by ARGENT 1973). Although BIZOT (in BIZOT, Pócs 1983: 31) synonymized it with *P. flagelliferum* Mitt., the julaceous habit of branches makes this species completely different from any other species of the genus *Pterobryon*. Even the generic position of *Pterobryon julaceum* cannot be definitely established at the present time, as all available material is sterile. Annotation by RO.

Meteoriaceae

31. **Papillaria flexicaulis** (Wils.) Jaeg. — WUS: 6951/R. *Papillaria flexicaulis* was not known previously from Africa or adjacent islands. It is a very widespread species in the whole tropical Asia and in Australasia, penetrating in the southern temperate belt, where it is known also from the Juan Fernandez Islands and from southern Chile (NOGUCHI 1976). The material from the Usambara Mountains agrees in all respects with *P. flexicaulis*



Fig. 4. West Usambara Mts. Baga II. F. R. Ericaceous heath on the isolated peak E of Kwagoroto summit, at 1900 m. Loc. 6956



Fig. 5. The interior of Mazumbai University Forest Reserve near "Lundgren's plot" at 1450 m alt. Submontane rain forest, locality 6962

I observed from different parts of Asia and Australasia. On the other hand, relationship between *P. africana* and *P. flexicaulis* badly needs a careful revision as these species are closely related. Annotation by RO.

32. *Pilotrichella ampullacea* (C. Müll.) Jaeg. — WUS: 6957/A, det. RO. Very widespread in tropical Africa and in the Mascarenes, EAB I, II, IV, V, VI.
33. *Aërobrydrium subpiligerum* (Hampe) Card. — EUS: 6947/P, 6948/B, det. RO. Lemurian species, widely distributed in the old crystalline mountains of East Africa, very rare in volcanic areas, EAB I, II, IV, V, VI.

Phyllogoniaceae

34. *Catagonium nitens* (Brid.) Card. — WUS: 6957/R, det. P. Southern temperate species occurring also in aequatorial mountains, in East Africa only within the area of old crystalline blocks, EAB I, V.

Neckeraceae

35. *Leptodon smithii* (Hedw.) Web. et Mohr var. *beccarii* (C. Müll.) Tong. — WUS: 6951/K, det. P. East African montane variety or species, EAB I, IV, VI.
36. *Pinnatella oblongifrondea* (Broth.) Broth. — WUS: 6950/D, det. RO. Widespread lowland and submontane species in tropical Africa, distributed from Cameroon to Tanzania, EAB I, IV, V.
37. *Porotrichum elongatum* (Welw. et Duby) Gepp — EUS: 6947/AC, det. RO. Very widespread tropical African species, EAB I, V — as *P. comorense* (DE SLOOVER 1983).



Fig. 6. Buttresses of an emergent *Newtonia buchananii* tree in the same plot as on Fig. 5. Habitat of *Cololejeunea tanneri* n. sp., which lives on filmy fern leaves epiphytic on the buttresses

Rigodiaceae

38. **Rigodium kilimandscharicum** (Broth.) Par. — WUS: 6954/F, det. P. East African montane species with tropical American affinities, EAB I, V, VI with map on p. 380.

Daltoniaceae

39. **Distichophyllidium africanum** Dem. et P. Varde — ULU: 6967/AM, det. P. A very rare East African montane species with tropical American affinities, EAB I, V.

Hypopterygiaceae

40. **Cyatophorella africana** (Dix.) Broth. — ULU: 6966/B, det. P. The only African species of an Indomalayan-Oceanian genus, EAB I, map: Pócs 1976: 96.

Brachytheciaceae

41. **Brachythecium afro-glareosum** (Broth.) Par. — WUS: 6950/A, det. RO. Fairly common East African montane species, from Tanzania to Ethiopia, EAB I, IV, V, VI.
 42. **Schimperella atro-thea** (P. Varde) P. Varde — WUS: 6950/C, det. RO. Rare East African montane species, new to the Usambaras, EAB IV, V, VI.
 43. **Rhynchostegiella holstii** (Broth.) Broth. — WUS: 6959/D, det. RO. Widespread East African montane species, EAB I, IV, V.



Fig. 7. West Usambara Mts. Shume-Magamba F. R. Tree fern stand in the "Kandele Kampala" saddle between Magamba and Mabweni, at 1900 m alt. Locality 6205



Fig. 8. West Usambara Mts. Shume-Magamba F. R. *Neorutenbergia usagarae* growing on a twig in the dry elfin forest of Magamba summit, at 2200 m alt. Locality 6954

Sematophyllaceae

44. *Trichosteleum borbonicum* (Bel.) Jaeg. — WUS: 6955/J, det. RO. Previously reported from mainland Africa only once (Usambara Mts., BROTHERUS 1897). Lemurian species.
45. *Trichosteleum mamillipes* Broth. — EUS: 6946/L, 6947/R, 6948/C, det. RO. Very widespread submontane and montane species of tropical Africa. EAB I, IV, V.

Hypnaceae

46. *Leucomium golungense* Gepp. — ULU: 6966/BE, det. P. Tropical African species, EAB I.
47. *Taxiphyllum gabonense* Broth. et P. Varde var. *plagiothecioides* P. Varde — EUS: 6947/T, det. RO. Rare lowland-submontane species distributed in tropical Africa, EAB I, V.
48. *Vesicularia galerulata* (Duby) Broth. — EUS: 6947/U, det. RO. Widespread submontane-montane species in South and East Africa and on the Mascarenes, EAB I, IV, V, VI.

Polytrichaceae

49. *Pogonatum usambaricum* (Broth.) Par. — EUS: 6947/O, det. RO. East African montane species, EAB I, IV, V.

Conclusions

The bryophytes discussed in the present paper and in the previous East African Bryophytes, VII (Pócs 1985) contribute to the high rate of endemism in the Usambaras. Even in the tropics it is quite exceptional, that obviously endemic species, not easily overlooked, like *Renauldia lycopodioides* or *Pterobryon julaceum*, are restricted to such a small area, together with endemic liverworts, as *Plagiochila angustitexta*, *Radula pseudoflaccida*, *Cladolejeunea aberrans*, *Cololejeunea amaniensis* and *C. tanneri*. These endemics, together with rare Lemurian and Palaeotropic species, represent high conservational value. In addition, some of the phytogeographically interesting bryophytes (*Dicranoloma billarderi*, *Neorutenbergia usagarae*) are conspicuous rain interceptors in the water catchment area of the Usambaras.

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STUDIES IN RONDELETIEAE, VIII

NUEVOS TAXA DEL GÉNERO RONDELETIA EN CUBA

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In this paper of the series authors publish the results of the taxonomic revision of the Cuban species of the genus *Rondeletia*. They distinguish, characterize and describe fourteen taxa new to science, ten new species, 2 new hybrids and 2 new subspecies. They are: *Rondeletia acunae* Borhidi et Fernandez sp. n., *R. combsioides* Fernandez et Borhidi sp. n., *R. convoluta* Fernandez et Borhidi sp. n., *R. galanensis* Fernandez et Borhidi sp. n., *R. lucida* Fernandez et Borhidi sp. n., *R. miraflorensis* Fernandez et Borhidi sp. n., *R. papayoensis* Fernandez et Borhidi sp. n., *R. peninsularis* Fernandez et Borhidi sp. n., *R. subcanescens* Fernandez et Borhidi sp. n., *R. toensis* Fernandez et Borhidi sp. n., *R. × incerta* Borhidi et Fernandez hybr. n., *R. × obscura* Borhidi et Fernandez hybr. n., *R. intermixta* Britt. ssp. *turquinensis* Fernandez et Borhidi, and *R. pachyphylla* Kr. et Urb. ssp. *myrtilloides* Fernandez et Borhidi.

Rondeletia acunae Borhidi et Fernandez sp. nova (Fig. 1)

Rami hornotini leviter angulati, adpresse sericeo-pilosi, veteriores teretes, glabrescentes, lenticellis oblongo-linearibus sparse dispositis. Stipulae triangular-subulatae, 2 mm longae, cum arista 2 mm longa, utrinque sericeo-pilosae. Folia 2-4 mm longe petiolata, oblanceolata vel elliptica, 0,7-2,0 cm longa et 0,3-1,2 cm lata, apice apiculata et 0,2-0,5 mm longe rigide mucronata, basi longe attenuata, lamina supra glabra et valde ruguloso-plicatula, subtus dense albo-sericea, nervo medio supra impresso, subtus prominenti, lateralibus utroque latere 2-3 supra impressis subtus prominentibus et dense reticulatis, margine revoluta, rigide coriacea.

Flores 5-meri, axillares, 1-2, plerumque solitarii; pedunculus 2-3 mm (in fructu usque ad 5 mm) longus, sericeo-pilosus. Bractee 0-2, simplices, lineares vel 3-lobulatae, 2-4 mm longae, lobulis linearibus, centralibus laterales 2-3-plo superantibus. Calycis tubus cum hypanthio brevis, depresso globosus, usque ad 2 mm in diam., manifeste 5-costulatus, sericeo-pilosus; lobi 5, lanceolato-subulati, superne flexuosi, valde inaequales, minores 2-3 mm, majores 5-6 mm longi, tubo corollino aequilongi vel paullo superantes, in fructibus valde decidui. Corolla 5-7 mm longa, tubus 5-6 mm longus, extus dense et retrorse albo-pilosus, intus glaber; lobi 5, semiorbiculares vel orbiculari-ovati, 2 mm longi et lati, dorso dense albo-pilosi, supra glabri. Capsula parva, globosa, leviter costulata, usque ad 3 mm in diametro, pilosula.

Holotypus: BORHIDI 30259 HAC; Cuba; prov. Holguín (Oriente), in fruticetis sempervirentibus serpentinosis ad Cerro de la Playa de Vaca, inter Yaguaneque et Moa. Leg.: A. BORHIDI, R. CAPOTE et Ramona OVIEDO. Isotypus: BP 574019.



Fig. 1. Isotype specimen of *Rondeletia acunae* Borhidi et Fernandez BP 574019. BORHIDI, CAPOTE and OVIEDO

Obs.: *Rondeletiae venosae* Wr. in Griseb. (e prov. Pinar del Rio, excl. *R. savannarum* Britt.) affinis, quae a specie nostra stipulis triangularibus emucronatis, foliis apice plerumque rotundatis, basi obtusis vel rotundatis, bracteis minutis, floribus 4-meris, lobulis calycinis linearibus brevioribusque, capsulis majoribus abunde differt. Altera species affinis, *R. savannarum* Britt. (incl. *R. holguinensis* Urb.) stipulis triangularibus, foliis obovatis, apice rotundatis, floribus 4-meris, lobis calycinis linearibus brevioribusque, capsula majore aequo modo discrepat.

Specimina examinata: ACUÑA 12762 HAC; Cuba, Prov. Holguin (Oriente), Vicinity of Moa, Playa de Vaca. Leg.: J. ACUÑA, 11. 4. 1945. US !.

Ramitas jóvenes ligeramente angulosas, apretado sericeo-pelosas, las adultas cilíndricas, glabrescentes con lenticelas lineal-alargadas, esparcidamente dispuestas. Estípulas triangular-subuladas, 2 mm de largo, con una arista de 2 mm de largo, sericeo-pelosas en ambas caras. Hojas con pecíolos de 2–4 mm de largo, sericeo-pelosos, el limbo oblanceolado de 0.7–2.0 cm de largo y 0.3–1.2 mm de ancho, apiculado en el ápice con un mucrón de 0.2–0.5 mm de largo,

largamente estrechado en la base, glabro y muy rugoso-plegado en el haz, densamente albosericoso en el envés; nervio medio hundido en el haz, prominente en el envés, los laterales 2-3 pares, hundidos en el haz, prominentes y densamente reticulados en el envés; el margen muy, revuelto, rigidamente coriáceo.

Flores 5-meras, axilares, 1-2, mayormente solitarias, pedunculo sericeo-peloso, de 2-3 mm de largo (hasta 5 mm en fruto). Bracteas 0-2, simples y lineales o 3-lobuladas, de 2-3 mm de largo, con lóbulos lineales; el lóbulo central 2-3 veces mas largo que los laterales, sericeo-peloso. Tubo del caliz con el hipantio corto, deprimido-globoso de hasta 2 mm de diámetro, marcadamente 5-acostillado, sericeo-peloso, lóbulos 5, lanceolado-subulados, muy desiguales, los menores de 2-3 mm, los mayores de 5-6 mm de largo, igual o mas largos que el tubo de la corola, muy caedizos en el fruto. Corola de 5-7 mm de largo, tubo de 5-6 mm de largo, densamente retrorso albo-peloso, lóbulos 5, semiorbiculares a orbicular-aovados, 2 mm de largo y ancho, densamente albo-peloso por el dorso, glabros por arriba. Capsula pequeña, de hasta 3 mm de diámetro, globosa, ligeramente acostillada, pelosita.

Holotipo: BORHIDI 30259 HAC; Cuba; prov. Holguín (Oriente); en charascales siempreverdes serpentinosos en el Cerro de la Playa de Vaca entre Yaguaneque y Moa. Col.: A. BORHIDI, R. CAPOTE y Ramona OVIEDO, 14. 9. 1970. Isotipo: BP 574019.

Ejemplares estudiados: ACUÑA 12762, HAC, US; Cuba; prov. Holguín (Oriente), Vicinity of Moa, Playa de Vaca. Col.: J. ACUÑA, 11. 4. 1945.

Afin a *Rondeletia venosa* Wr. in Griseb. (de la provincia Pinar del Río) la que difiere de nuestra especie en tener estípulas triangulares, no mucronadas, hojas mayormente redondeadas en el ápice, obtusas o redondeadas en la base, bracteas muy pequeñas, flores 4-meras, lóbulos del cáliz lineares y mas cortos y capsula mas grande. Otra especie cercana es *R. savanarum* Britt. (incluida *R. holguinensis* Urb.), que se distingue por tener estípulas triangulares, hojas obovadas, redondeadas en el ápice, flores 4-meras, lóbulos del cáliz lineares y mas cortos, cápsulas mas grandes.

***Rondeletia combsioides* Fernandez et Borhidi sp. nova (Fig. 2)**

Rami teretes, hornotini pilosi, veteriores glabri. Stipulae deltoideo-apiculatae, sericeae, 3-4 mm longae. Folia 2-6 mm longe petiolata, petiolis hirsutis suffulta, elliptica, oblongo-elliptica vel oblongo-oblancoolata, 1,5-5,5 cm longa et 0,5-2 cm lata, apice rotundata vel obtusa, breviter apiculata, basi longe cuneata; nervo medio subtus prominenti, lateralibus utroque latere 3-4 apicem versus arcuatis, strigillosis, supra tenuiter impressis, subtus dense reticulatis et prominulis, lamina foliorum juvenilium utrinque sericeo-pilosa, senescentium supra glabra vel glabrescens, subtus sparse pilosula, ad nervos sericea, margine plana, chartacea.

Inflorescentiae axillares, 3-4-florae, hirsutae; pedunculus 1-2 mm longus, pedicelli subnulli. Bractaeae 2, triangulares, 1 mm longi, liberi, hirsuti. Calycis tubus subglobosus, 1,5-2 mm longus, hirsutus; lobi 4-5, breviter triangulares vel deltoidei, 0,5-1 mm longi, apice acuti vel obtusi, sub anthesi reflexi. Corolla non visa. Capsula globosa, lobis calycinis persistentibus coronata, tomentosa, 2-3,5 mm in diam. Semina triangulari-oblonga, 0,5-1 mm longa, exalata, superficie rugosa.

Holotypus: EKMAN 15135 S! Cuba; Prov. Holguín (Oriente); Sierra de Nipe, in limestone hills at Río Jimbambay. Col.: EKMAN, 19. 9. 1922. Isotypi: GH, NY, US.



Fig. 2. Holotype specimen of *Rondeletia combsioides* Fernandez et Borhidi in S; EKMÁN 15135

Obs.: Verisimiliter *Rondeletiae combsii* Greenm. affinis, quae a specie nostra foliis apice plerumque acutis, floribus consenquenter 4-meris, lobis calycinis anguste triangularibus vel lanceolatis acutis, 1.5–2.5 mm longis, in fructu deciduis differt.

Ramitas cilindricas, pelosas cuando jóvenes, glabras con la edad. Estípulas deltoideo-apiculadas, de 3–4 mm de largo, sericeas. Hojas pecioladas, pecíolos pubérulos de 2–6 mm de largo. Hojas elípticas, oblongo-elípticas u obovado-oblongas de 1.5–5.5 cm de largo y 0.5–2 cm de ancho, las jóvenes pelosas en ambas caras, las adultas glabras a glabrescentes por el haz, pelositas por el envés, estrigilosas en los nervios. Nerviación poco aparente en el haz, marcada en el envés, los laterales en pares de 3–4 arqueadas hacia el ápice; el margen plano, limbo cartáceo el ápice redondeado a obtuso brevemente apiculado, la base largamente estrechada, cuneada.

Inflorescencias axilares, 3–4-floras, hirsutas; pedunculos de 1–2 mm de largo, hirsuto, pedicelos muy cortos, subnulos. Brácteas 2, triangulares, de 1 mm de largo, libres, hirsutas. Cáliz subgloboso, hirsuto, de 1.5–2 mm de largo, lóbulos 4–5, pelosos en ambas caras, triangulares o deltoideos muy cortos de 0.5–1 mm de largo con el ápice agudo u obtusito, reflejos

en la floración. Cápsula globosa, de 2–3.5 mm de diámetro, tomentosa, coronada por los lóbulos persistentes del cáliz. Semillas diminutas, rugulosas, no aladas, de forma variada, angulosas a veces disciformes, de 0.5–1 mm de largo. Corola no vista.

Holotipo: EKMAN 15135 S! Cuba; Prov. Holguín (Oriente); Sierra de Nipe, en las lomas calizas del Río Jimbambay. Col.: EKMAN 19. 9. 1922. **Isotipos:** GH, NY, US.

Afin a *Rondeletia combsii* Greenm. (de Cuba Occidental), la que difiere de nuestra especie en tener hojas mayormente agudas en ápice, peloso-tomentosas en ambas caras, flores consecuentemente 4-meras, pedunculos mas largos, lóbulos del cáliz estrechamente y oblongo triangulares a lanceolados, agudos, de 1.5–2.5 mm de largo, deciduos en el fruto, cápsulas mas grandes.

***Rondeletia convoluta* Fernandez et Borhidi sp. nova**

(Syn.: *Rondeletia bicolor* sensu Alain Fl. de Cuba, 1962: 51. p.p. non Britt.)

Rami hornotini quadranguli, pubescentes. Stipulae interpetiolares triangulari-subulati usque ad 4 mm longi. Folia 2–3 mm longe petiolata, petiolis sericeis suffulta, oblanceolata vel oblanceolato-elliptica, 1.5–2.5 cm longa et 0.5–1 cm lata, apice obtusa, rariter apiculata, basi attenuata et in petiolum contracta, satis longe cuneata; nervo medio supra impresso, lateralibus utroque latere 4–5, supra impressis, subtus prominentibus; lamina supra glabra, nitida, valde rugosa, subtus albo-tomentosa vel lanuginosa, margine valde revoluta, coriacea.

Flores axillares solitarii, 5-meri; pedunculi 3–4 mm longi sericei, bractee 2 sericeo-pilosae, lineares, 3–4 mm longae, basi ovarii involucriformiter connatae. Hypanthium sericeum, tubus calycis 5-angulosus, superne 2–2.5 mm late dilatatus, lobi calycis 5, lineari-lanceolati, 4–5 mm longi, inter sese in 1/4 infimum connati, apice obtusi vel rotundati, flexuosi. Corollae tubus 5–6 mm longus retrorse pilosus et albo-tomentosus, sub fauce manifeste dilatatus, lobi 5, orbicular-obovati, intus tomentosi. Capsula globosa, 3–3.5 mm in diametro, sericeo-pilosa.

Ramitas cuadrangulares, pubérulas. Estípulas triangular-subuladas, de 4 mm de largo. Hojas pecioladas con pecíolos sericeos de 2–3 mm de largo, limbo oblanceolado a oblanceolato-elíptico, de 1.5–2.5 cm de largo y 0.5–1 cm de ancho, glabro, muy rugoso y algo brillante por el haz, el envés albo-peloso a tomentoso, el margen muy revuelto, coriáceo. Ápice del limbo obtuso, en algunas hojas apiculado, la base estrechada hacia el pecíolo, cuneada. Nervio medio y los laterales de 4–5 pares hundidos en el haz, prominentes en el envés.

Flores solitarias, axilares, 5-meras; pedunculos de 3–4 mm de largo, sericeos, brácteas 2, lineales, de 3–4 mm de largo, connados en la base del ovario formando un involucro. Hipantio sericeo, tubo del cáliz 5-anguloso, alargado sobre el ovario, ensanchado hacia arriba de 2–2.5 mm de largo, lóbulos 5, lineales, de 4–5 mm de largo, connados en la base en un 1/4 de su longitud, el ápice obtuso o redondeado, flexuoso; tubo de la corola de 5–6 mm de largo, retrorso-peloso, lóbulos 5 albo-tomentosos. Capsula globosa, de 3–3.5 mm de diámetro, sericeo-pelosa.

Holotypus: A. LUNA 865 HAC; Cuba; Santa Clara; Lomas de Banao. Agosto–Octubre, 1920.

Specimina examinata: LEÓN 8069. S, NY; Sta Clara. Banao Mts. Loma del Brollo. Agosto 2, 1918.

Obs.: Cum *Rondeletia bicolori* Britt. (e Sierra de Banao) confusa, quae a specie nostra foliis ellipticis, majoribus, supra in sicco nigricantibus, margine plerumque planis, floribus verisimiliter 4-meris satis longe distat. Re vera species proxima *Rondeletia acunae* Borhidi et Fernandez videtur (e Oriente, Moa), quae a specie nostra foliis apice mucronatis, nervis lateralibus utroque latere 2-3, lobis calycinis valde inaequalibus, usque ad basem liberis, lobis corollinis intus glabris, capsulis minoribus differt.

Esta especie habia sido confundida varias veces con la *Rondeletia bicolor* Britt. que ha sido conocida sólo de la colección típica, y que difiere en tener hojas elípticas, mayores, negruzcas en el haz cuando secas, margen plano y flores probablemente 4-meras. La especie proxima realmente es la *Rondeletia acunae* Borhidi et Fernandez (de Oriente, Moa), que se distingue en tener hojas mucronadas en el ápice, con margen revuelto, nervios laterales 2-3 pares, lóbulos del cáliz muy desiguales, libres hasta la base, lóbulos de la corola glabros por dentro y capsulas menores.

***Rondeletia galanensis* Fernandez et Borhidi sp. nova (Fig. 3)**

Frutex; ramuli hornotini ferrugineo-hirsuti vel pubescentes, veteriores glabri, internodiis 3-5 cm longis suffulti. Stipulae late triangulares, acuminatae, 1,5-2 mm longae, pilosae. Folia 3-5 mm longe petiolata, late elliptica vel suborbicularia, rariter ovata vel oblango-ovata, 4-7,5 cm long et 3-5 cm lata, apice obtusa vel rotundata, basi rotundata vel subcordata, leviter obliqua; nervo medio supra impresso, subtus prominenti et apicem versus angustato, lateralibus utroque latere 3-5, in angulo 60-80° abeuntibus supra obsoletis subtus conspicuis at ante marginem anastomosantibus, reticulatis. Lamina utrinque glabra, nitida, subtus ad nervum medium strigillosa, margine plana vel paullo recurva, crasse coriacea.

Inflorescentia cymoso-paniculata axillaris et terminalis, oblonga usque ad 15 cm longa, ramulis crassis erectisque ferrugineo-pilosis, multiflora. Pedunculus 3-5 cm longus, pedicelli 0,7-1,3 cm longi, erecti. Calycis tubus globosus, 0,9-1,4 mm in diametro, hirsutus, lobi 5, orbicular-spathulati, basi valde attenuati, 1,5-2 mm longi, hypanthio aequilongi vel longiores, pubescentes. Bracteae 2, spathulatae, 2 mm longae, puberulae, bracteolae 2, lineares, liberae, 2 mm longae, puberulae. Corolla non visa. Capsula globosa, pubescens, lobulis calycinis persistentibus coronata, 4-5 mm in diametro. Semina numerosa, diminuta, utrinque attenuata et stipitata, rugosa et reticulato-foveolata.

Holotypus: ALAIN 3698 HAC!; Cuba; Prov. Guantánamo (Oriente). Bosque humedo cerca de la cumbre del Pico Galán, Sierra del Frijol, La Alegria, Toa; alt. aprox. 1100 m.s.m. 1. 1. 1954. Col.: ALAIN. Isotypus: NY.

Specimina examinata: ALAIN 3615 HAC, NY; Prov. Guantánamo (Oriente). Serpentine barrens, Peña Prieta La Magdalena, Toa. Alt. aprox. 600 m.s.m. Col.: ALAIN 30. 12. 1953.

Obs.: *Rondeletia alaternoidi* A. Rich. affinis, quae a specie nostra foliis ellipticis vel oblongo-ellipticis, inflorescentiis brevibus et paucifloris, pedunculis pedicellisque tenuibus et flexuosis, lobulis calycinis oblongo-spathulatis foliaceis, capsulis minoribus differt. Altera species huius generis *R. myrtacea* Standl. in Britt. foliis minoribus atque inflorescentiis hypanthioque glabris distinguitur. *R. grandisepala* Alain foliis cordiformibus et inflorescentiis capitatis breviter pedunculatis, *R. lucida* Fernandez et Borhidi foliis utrinque lucidis et valde revolutis, inflorescentiis perfecte glabris differunt.

Arbusto; ramitas ferrugineo-hirsutas a pubescentes, glabras con la edad. Entrenudos de 3-5 cm de largo. Estípulas anchamente triangulares, acuminadas de 1.5-2 mm de largo,

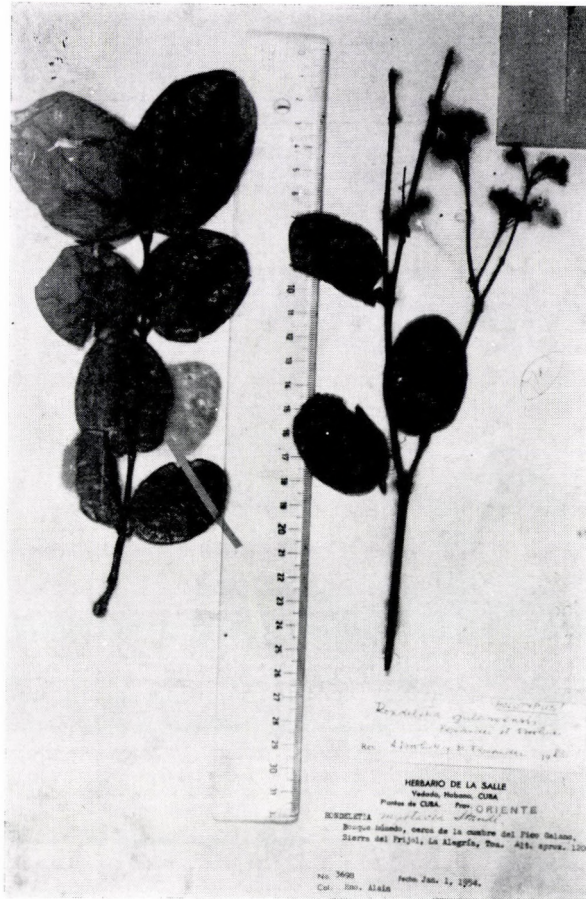


Fig. 3. Holotype specimen of *Rondeletia galanensis* Fernandez et Borhidi in HAC; ALAIN 3698

pelositas. Pecíolos cortos, de 3–5 mm de largo, glabrescentes. Hojas anchamente elípticas a suborbiculares u oval-oblongas a ovales de 4–7.5 cm de largo y 3–5 cm de ancho, el ápice obtuso a redondeado, la base redondeada a subacorazonada, algo inequilátera; nervio medio hundido en el haz, prominente y estrechado hacia el ápice en el envés, los laterales de 3–5 pares salen en un ángulo de 60–80°, subnulos en el haz, aparentes y anastomosados antes del margen por el envés con una reticulación visible. Lámina de las hojas glabras y brillantes en ambas caras, estrigosas en el nervio medio del envés.

Inflorescencia cimoso-paniculada compuesta y alargada de hasta 15 cm, con ramitas gruesas y erectas, ferrugíneo-pilosa y multiflora. Pedunculos de 3–5 cm, pedicelos de 0.7–1.3 cm de largo, todos erectos. Tubo del cáliz globoso de 0.9–1.4 mm de diámetro, hirsuto; lóbulos 5, orbicular-espátulados, muy estrechados en la base, de 1.5–2 mm de largo, iguales o mas largos que el hipantio, pubescentes. Brácteas 2, espátuladas, de 2 mm de largo, pubérulas; bractéolas 2, lineares, libres, de 2 mm de largo, pubérulas. Corola no vista. Cápsula globosa, pubescente, con lóbulos del cáliz persistentes, de 4–5 mm de diámetro. Semillas numerosas, muy pequeñas, estrechadas en ambos extremos, rugosas y reticulado-foveoladas.

Holotipo: ALAIN 3698 HAC! Cuba; prov. Guantánamo (Oriente); bosque húmedo cerca de la cumbre del Pico Gálan, Sierra de Frijol, La Alegria, Toa, alt. aprox. 1100 m.s.m. Col.: ALAIN l. l. 1954. Isotipo: NY.

Ejemplares examinados: ALAIN 3615 HAC, NY; prov. Guantánamo (Oriente); serpentine barrens; Pena Prieta, La Magdalena, Toa. Alt. 600 m.s.m. Col.: ALAIN 30. 12. 1953.

Afin a *Rondeletia alaternoides* A. Rich. ssp. *alaternoides* que se distingue en tener hojas elípticas u oblongo-elípticas, inflorescencias cortas y paucifloras, pedunculos y pedicelos delgados y flexuosos, lóbulos del cáliz oblongo-espátulados, cápsulas menores; la *R. myrtacea* Standl. in Britt. además de estas características difiere en tener hojas mas pequeñas e inflorescencias glabras. Otra especie cercana, *R. grandisepala* Alain se distingue en tener hojas acorazonadas subsentadas con nerviación mas acentuada en ambes caras, inflorescencias capitadas, corto-pedunculadas. Especie proxima *R. lucida* Fernandez et Borhidi se distingue en tener hojas oblongo-elípticas, muy revolutas y lustrosas en el haz, inflorescencias completamente glabras.

***Rondeletia miraflorensis* Fernandez et Borhidi sp. nova**

Frutex 1-2 m altus. Rami hornotini 4-anguli, dense breviterque retrorso-pilosi, internodiis 0,2-1 cm longis, veteriores teretes, longitudinaliter striati et transversaliter breviterque fissurati. Stipulae late ovatae vel semiorbiculares, 1-1,5 mm longae, basi usque ad dimidium annuliformiter connatae, apice rotundatae, dorso e basi dilatato 0,3-0,5 mm longe mucronatae, dense puberulae. Folia obovata vel orbiculari-obovata vel suborbicularia, 5-10 mm longa et 4-8 mm lata, 1 mm longe petiolata, vel sessilia, apice rotundata et plerumque breviter apiculata, basi breviter attenuata vel obtusa, rariter rotundata; nervo medio supra impresso, subtus crasse prominenti, lateralibus utroque latere 2-3 supra plerumque nullis, subtus arcuatis et ante marginem conjunctis, saepe inconspicuis, rariter anastomosantireticulatis; lamina supra glabra vel minutissime pilosa, nitida, valde plicata, subtus tomento albo dense coperta, ad nervos pilis brevibus sursum directis sericea, valde coriacea.

Flores 4-meri, in axillis foliorum solitarii, sessiles vel subsessiles. Prophylla triangularia vel ovata, apice acuta, 1-3 mm longa, basi involucriformiter connata, dense tomentosa. Hypanthium leviter 4-angulum, 1-1,5 mm in diametro, tomentosum, lobi calycis 4, lineari-ovati vel lineari-spathulati, sub apice plerumque leviter dilatati, 2,5-3,5 mm longi, apice acuti vel obtusi, basi 1 mm longe connati ceterum liberi. Corollae tubus 7-8 mm longus, extus retrorse hirsutus, intus glaber, lobi 4, suborbiculares vel obovati, 1,5-2,5 mm longi, utrinque tomentosuli. Stamina 4, subsessilia, in fauce corollae inserta, antherae oblongae vel lineares, 2 mm longae, insertae. Stylus basi leviter puberulus, in flore longistylus 8-9 mm longus, brevissime exsertus. Capsula 1-3 mm longe pedunculata, pedunculo 4-angulo, retrorse hirsuto, globosa, puberula, 4-6 mm in diametro.

Holotypus: ALAIN 963 HAC; Cerro de Miraflores, July, 1949. Leg.: ALAIN et CLEMENTE. Isotypus: NY.

Specimina examinata: LEÓN 21145, Cerro de Miraflores, Cananova, July, 1942. GH, HAC, NY, US; — idem, LEÓN 21163, GH, HAC; — idem, BP 574059 et 574060, leg.: BORHIDI, CAPOTE et OVIEDO, Sept. 11. 1974.

Obs.: *Rondeletiae steiophyllae* Urb. (e Sierra del Cristal) affinis, quae a specie nostra foliis orbiculari-ovatis, juvenilibus supra arachnoideo-tomentulosis, subtus ad nervos retrorse setulosis, inter nervos arachnoideo-tomentosis, floribus 3-4 mm longe pedunculatis, prophyllis

liberis, spathulatis vel orbicularibus, apice rotundatis, hypanthio globoso differt. Altera species proxima *R. hypoleuca* Griseb. (e Baracoa) foliis apice non apiculatis, nervis lateralibus 3-4, supra conspicuis, subtus valde prominentibus, bracteis semiorbicularibus, apice rotundatis, involueralibus, lobis calycinis triangularibus, 1-2 mm longis distinguitur.

Arbustos de 1-2 m de alto; ramitas 4-angulosas, brevemente retrorso-pelosas con entrenudos de 0.2-1 cm de largo, las adultas cilíndricas, longitudinalmente estrigosas con fisuras transversales cortas. Estipulas anchamente aovadas o semiorbiculares de 1-1.5 mm de largo, tomentosas, connadas en un anillo en la base, el ápice redondeado, con un mucrón de 0.3-0.5 mm de largo, saliendo del dorso. Hojas obovadas u orbicular-obovadas o sub-orbiculares, sésiles o con un peciolo de hasta 1 mm de largo, limbo de 5-10 mm de largo y 4-8 mm de ancho, redondeado y mayormente apiculado en el ápice, brevemente estrechado, obtuso o redondeado en la base; nervio medio hundido en el haz, engrosado y prominente en el envés, los laterales de 2-3 pares inconspicuos en el haz, encorvados y anastomosados antes del margen, por lo común poco conspicuos, a veces reticulados en el envés; glabro u muy diminutamente peloso, brillante y muy plegado en el haz, densamente albo-tomentoso con pelos sericeos cortos dirigidos hacia el ápice en los nervios del envés, muy coriáceo.

Flores 4-meras, solitarias en las axilas, sésiles o subsésiles. Brácteas 2, triangulares o aovadas de 1-3 mm de largo, agudas a obtusitas en el ápice, connadas en un involuero tomentoso en la base. Hipantio 4-ángulo de 1-1.5 mm diámetro, tomentoso; lóbulos del cáliz 4, lineal-aovados o lineal-espatulados, ligeramente ensanchados debajo del ápice, connados en la base en un tubo de 1 mm de largo sus partes libres de 2.5-3.5 mm de largo, agudos u obtusos en el ápice. Corola rosada, tubo de 7-8 mm de largo, retrorso-hirsuto por fuera, glabro por dentro, lóbulos 4, suborbiculares u obovados de 1.5-2.5 mm de largo, tomentosos en ambas caras. Estambres 4, subsésiles, insertos en la garganta del tubo de la corola, anteras oblongas a lineales, de 2 mm de largo. Estilo peloso en la base, de 8-9 mm de largo, poco exerto. Cápsula globosa de 4-6 mm de diámetro, puberula, pedunculo fructificado 4-ángulo, retrorso-peloso, 1-3 mm de largo.

Holotipo: ALAIN 963 HAC; Prov. Holguín (Oriente), Moa: Cerro de Miraflores; col.: ALAIN y CLEMENTE, julio 1949. Isotipo: NY.

Ejemplares estudiados: LEÓN 21145, Cerro de Miraflores, Cananova; julio, 1942. GH, HAC, NY, US; — idem, LEÓN 21163, GH, HAC; — idem, BP 574059 y 574060, col.: BORHIDI, CAPOTE y OVIEDO, Sept. 11. 1974.

Afin a *Rondeletia steiophylla* Urb. (de la Sierra del Cristal), pue difiere de nuestra planta en tener hojas orbicular-aovadas, tomentosulas en el haz, cuando jóvenes, retrorso-estrigilosas en los nervios del envés y araneoso-tomentosas entre los nervios; flores con pedunculo de 3-4 mm de largo, brácteas libres, espatuladas u orbiculares, redondeadas en el ápice, hipantio globoso. Otra especie cercana, *R. hypoleuca* Griseb. (de Baracoa) difiera en tener hojas no apiculadas en el ápice, nervios laterales de 3-4 pares, conspicuos en el haz, prominentes en el envés, brácteas suborbiculares, redondeadas en el ápice, connadas, lóbulos del cáliz triangulares de 1-2 mm de largo.

***Rondeletia* × *incerta* Borhidi et Fernandez hybr. nova (Fig. 4)**

(*Rondeletia miraflorensis* × *subcanescens*)

Rami hornotini sericei, rariter retrorso-pilosi; stipulae late triangulares, 1-2 mm longae, apice breviter acuminatae et mucronatae, sericeo-pilosae. Folia elliptica vel obovato-elliptica vel oblonga, 1-2 mm longe petiolata, 0.5-1.4 cm longa et 0.3-0.7 cm lata, supra

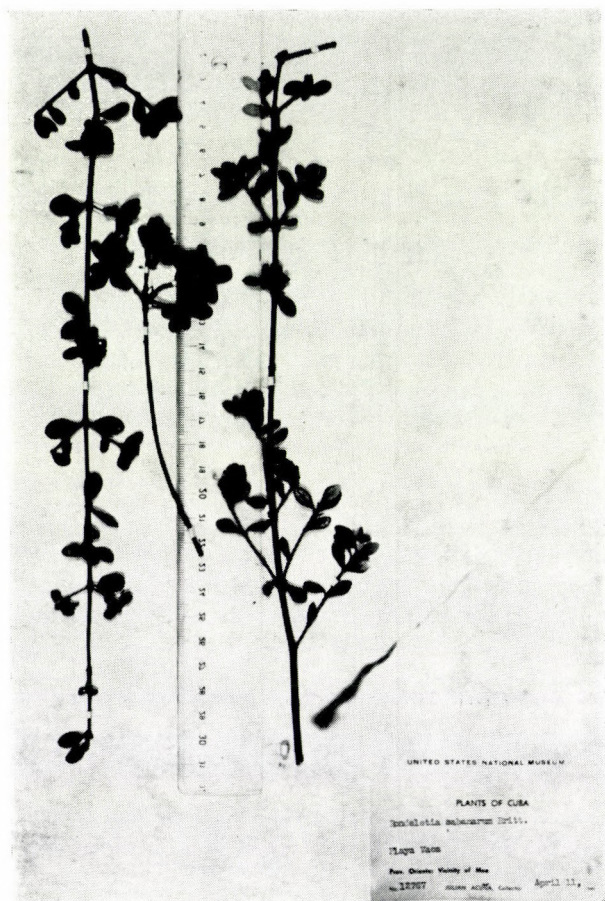


Fig. 4. Isotype specimen of *Rondeletia* \times *incerta* Borhidi et Fernandez in US 1881313; ACUÑA 12767

glabra vel glabrescentia, nitida et rugosa, subtus albo tomentosa, apice rotundata vel obtusa, brevissimo, mucronata, basi cuneata, margine saepe ciliata, revoluta, coriacea; nervo medio supra impresso, subtus crasse prominente et canaliculata, lateralibus supra nullis, subtus utroque latere 2–3, bene conspicuis et leviter impressis, posterius manifeste reticulatis.

Flores 4-meri solitarii, terminales et axillares; pedicelli 1–2 mm longi (in fructu usque ad 3 mm), sericeo-pilosi. Bracteae 2, liberae, suborbiculares, apice rotundatae, 3–4 mm longae, bracteolae 2, triangulares, liberae, 1–2 mm longae. Calycis tubus leviter 4-angulatus, 2–3 mm longus, sericeo-pilosus, lobi 4, lineari-obovati, vel brevissime spathulati, longitudine varii, 2–4 mm longi, utrinque albo-sericei. Capsula globosa, superficie rugulosa, 3–4 mm longa, albo-sericea.

Ramitas jóvenes sericeas, algunas retrorso-pelosas. Estípulas de 1–2 mm de largo, anchamente triangulares con el ápice corto acuminado, sericeas. Pecíolos de 1–2 mm de largo, sericeo-pelosos. Hojas elípticas u obovado-elípticas u oblongas, de 0.5–1.4 cm de largo, por 0.3–0.7 cm de ancho, glabras a glabrescentes, brillantes y rugosas en el haz, el envés albo-

tomentoso, el ápice redondeado a obtuso muy brevemente mucronulado, estrechando hacia la base cuneada. El margen a menudom ciliado, revoluto. Limbo coriáceo. Nervio medio hundido en el haz, los laterales nulos, cuando aparentes también hundidos. Por el envés el nervio medio aparente y algo acanalado, hacia el ápice se va estrechando y tiende a desaparecer, prominulo, los laterales de 2-3 pares hundidos y bien marcados, con la edad se acentua la reticulación.

Flores 4-meras, solitarias, terminales y axilares, pedicelos cortos de 1-2 mm de largo (hasta 3 mm en fruto), sericeo-pelosos. Brácteas 2, libres, de 3-4 mm de largo, suborbiculares, redondeadas en el ápice, bracteolas 2, libres, triangulares de 1-2 mm de largo. Cáliz algo anguloso de 2-3 mm de largo, albo-sericeo, lóbulos 4, lineal-obovados, algunos muy brevemente espatulados, 2-4 mm de largo, desiguales, albo-sericeos en ambas caras. Cápsula globosa de 3-4 mm de diámetro, pelosita, rugulosa.

Holotypus: ACUÑA 12767. HAC; Prov. Holguín; Pinares de Moa, leg. J. ACUÑA, 11. 4. 1945. **Isotypus:** US 1881313.

Rondeletia intermixta Britt. (Fig. 5.)

ssp. *intermixta*:

foliis ellipticis, oblongo-ellipticis vel oblongo-lanceolatis, 3-11 cm longis et 1,2-4 cm latis apice acuminatis et acutis, basi longe attenuatis et cuneatis, nervis lateralibus utroque latere 6-8, subtus dense sericeo-pubescentibus, inflorescentiis plurifloris pedunculis usque ad 1,5 cm longis, calyce dense strigilloso-hirsutis, 4-5-lobulatis, tubo corollino 6-7 mm longo.

Hojas elípticas, elíptico-oblongas u oblongo-lanceoladas, 3-11 cm de largo y 1.2-4 cm de ancho, acuminadas y agudas en el ápice, largamente estrechadas y cuneadas en la base, densamente sericeo-puberulas en el envés nervios laterales de 6-8 pares; inflorescencias plurifloras con pedunculos de hasta 1.5 cm de largo, cáliz densamente estrigiloso-hirsuto, 4-5-lobulado, tubo de la corola de 6-7 mm de largo.

Holotypus: SHAFFER 9039; Cuba; prov. Oriente, Gran Piedra. NY. **Isotypi:** HAC, US.

Area: Cuba; Sierra Maestra, Cordillera de la Gran Piedra, entre 800 y 1200 m.s.m.

ssp. *turquinensis* Fernandez et Borhidi ssp. nova (Fig. 6)

A typo differt: foliis minoribus et ovatis, basi obtusis, truncatis vel rotundatis, 1,5-5 cm longis et 0,5-2,4 cm latis, subtus sparse pilosis vel glabrescentibus, nervis lateralibus utroque latere 4-6, supra obsoletis; inflorescentiis axillaribus cymoso-racemosis paucifloris, breviter (0,5-1 cm longe) pedunculatis, minus breviusque hirsuto-tomentosis, floribus 5-meris, tubo corollino 5-6 mm longo.

Difiere del tipo en tener hojas menores y ovadas, obtusas, truncadas o redondeadas en la base, de 1.5-5 cm de largo y 0.5-2.4 cm de ancho, esparcidamente pelosas a glabrescentes en el envés, nervios laterales de 4-6 pares, poco conspicuos en el haz; inflorescencias axilares cimoso-racemosas paucifloras menos y corto-pubérulas, con pedunculos de 0.5-1 cm de largo, flores 5-meras, tubo de la corola de 5-6 mm de largo.



Fig. 5. Holotype specimen of *Rondeletia intermixta* Britt. in NY, SHAFER 9039

Holotypus: EKMAN 14599 S!; Cuba; Prov. Granma (Oriente); Sierra Maestra, in steep rocks of Loma Regino, alt. cca 1700 m.s.m.l. Col.: EKMAN 24. 7. 1922.

Conocida solo de la colección típica.

***Rondeletia lucida* Fernandez et Borhidi sp. nova (Fig. 7)**

Frutices ramulis teretibus glabris, lenticellis elongatis longitudinaliter striatis, internodiis longitudine variis (1–5 cm longis). Stipulae triangulares, apice acuminatae, 1–2 mm longae, extus glabrae, intus pilosae. Folia 2–7 mm longe petiolata, petiolo glabro, lamina oblongo-elliptica vel elliptica, 1,5–7 cm longa et 0,7–3 cm lata, apice obtusa, rotundata vel levissime emarginata, basi obtusa vel breviter rotundata, nervo medio supra impresso, subtus crasse prominenti, lateralibus utroque latere 3–7, supra leviter prominulis et manifeste den-



Fig. 6. Holotype specimen of *Rondeletia intermixta* Britt. ssp. *turquinensis* Fernandez et Borhidi in S. EKMAN 14599

seque reticulatis, in foliis veterioribus levissime impressis, subtus leviter prominulis reticulo obsoleto vel inconspicuo; lamina rigide coriacea, utrinque lucida et glaberrima, subtus dense punctulato-muricata, margine valde revoluta.

Inflorescencia cymoso-paniculata, usque ad 10 cm longa, erecta, multiflora, glabra. Pedunculi erecti, 2–3 cm longi, pedicelli 0,5–1,5 cm longi. Tubus calycis cum ovario globosus, leviter angulatus, 1,5–2 mm in diametro; lobi 5, stipitato-orbiculari-spathulati, crassi, glabri vel glabrescentes, 1,5–3 mm longi. Bracteolae liberae, lineares, 2–2,5 mm longae, glabrae; bractee 2, late spathulatae, usque ad 5 mm longae, glabrae. Corolla non visa. Capsula depresso globosa, 4–7 mm in diametro, glabra, lobulis calycinis incrassatis persistentibusque coronata, loculicide dehiscens. Semina numerosa, diminuta, 1–1,5 mm longa, utrinque attenuata, reticulata et foveolata.

Holotypus: ALAIN 3453 HAC! Cuba. Prov. Guantánamo (Oriente). Sierra de Moa, charrascales serpentinosa en la cresta en la alt. 900 m. aprox. Col.: ALAIN, 25. 7. 1953. Isotypi: GH, NY.



Fig. 7. Holotype specimen of *Rondeletia lucida* Fernandez et Borhidi in HAC; ALAIN 3453

Specimina examinata: ALAIN 5795 p.p. NY. Cuba; Prov. Holguin (Oriente); Sierra del Cristal, on slopes of El Cristal, near the crest; alt. 1000–1200 m.a.s.l. Col.: ALAIN, ACUÑA and LOPEZ FIGUEIRAS, 2–7. 4. 1956.

Obs.: *Rondeletiae galanensi* Fernandez et Borhidi affinis, quae a specie nostra foliis late ovatis, basi rotundatis vel subcordatis, supra non reticulato venosis, inflorescentiis ferrugineo-puberulis differt.

Arbusto, ramitas, cilindricas, estriadas con lenticelas alargadas, glabras; entrenudos de 1–5 cm de largo. Estípulas triangulares, apiculadas en el ápice, de 1–2 mm de largo, glabras por fuera, pelosas por dentro. Hojas con pecíolo glabro de 2–7 mm de largo; limbo de las hojas oblongo-elíptico o elíptico, de 1.5–7 cm de largo y 0.7–3 cm de ancho, obtuso, redondeado o poco emarginado en el ápice, obtuso o brevemente redondeado en la base; nervio medio hundido en el haz, engrosado y prominente en el envés, los laterales de 3–7 pares ligeramente prominulos en el haz y marcadamente reticulados, o poco hundidos en las hojas mas adultas; poco prominulos en el envés con reticulación obsoleta o inconspicua; el limbo rigidamente

coriáceo, brillante y glabro en ambas caras, densamente muricado-punteado en el envés el margen muy revolutu.

Inflorescencia axilar y terminal, cimoso-paniculada, de hasta 10 cm de largo, erguida, multiflora y glabra. Pedunculo recto, de 2–3 cm de largo, pedicelos de 0.5–1.5 cm de largo. Tubo del cáliz con el ovario globoso, ligeramente anguloso, de 1.5–2 mm de diámetro; lóbulos 5, estipitado orbicular-espatulados, gruesos, coriáceos, glabros o glabrescentes, de 1.5–3 mm de largo. Bracteolas libres, lineares de 2–2.5 mm de largo, glabras, brácteas 2, anchamente espatuladas, de hasta 5 mm de largo, glabras. Corola no vista. Cápsula deprimido-globosa de 4–7 mm de diámetro, glabra, coronada por los lóbulos del cáliz engrosados y persistentes. Semillas numerosas, diminutas de 1–1.5 mm de largo, estrechadas en ambos extremos, reticuladas y foveoladas.

Holotipo: ALAIN 3453 HAC; Cuba; Prov. Guantánamo (Oriente); Sierra de Moa, charrascales serpentinosos en la cresta en la alt. 900 m.s.n.m. aproximadamente. **Col.:** ALAIN 25. 7. 1953. **Isotipos:** GH, NY.

Ejemplares estudiados: ALAIN 5795 p.p. in NY; Cuba; Prov. Holguín (Oriente); Sierra del Cristal, on slopes of El Cristal near the crest; alt. 1000–1200 m.a.s.l. **Col.:** ALAIN, ACUÑA and LÓPEZ FIGUEIRAS 2–7. 4. 1956.

Afin a *Rondeletia galanensis* Fernandez et Borhidi que se distingue en tener hojas anchamente ovales, redondeadas o subacorazonadas en la base, no reticulado venosas en el haz, inflorescencias ferruginoso-pubérrulas, cáliz y fruto pelosos.

***Rondeletia* × *obscura* Borhidi et Fernandez hybr. nova**
(*Rondeletia paucinervis* × *R. plicatula*)

Frutex; rami tortuosi, hornotini pilosi, veteriores scabrosi, teretes, cortice transversaliter fisso. Stipulae late triangulares usque ad 1 mm longae, ad ramos hornotinos densissime dispositae, apice mucronatae, basi breviter connatae, utrinque sericeo-strigillosae. Folia apice ramorum dense conferta, 1–2 mm longe petiolata, elliptica vel suborbicularia, rariter obovata, 3–9 mm longa et 2–5 mm lata, apice rotundata vel obtusa, saepe mucrone reflexo suffulta, basi attenuata vel obtusa, nervo medio supra profunde impresso, subtus crasse prominenti, lateralibus utroque latere 2–3, supra inconspicuis, subtus prominulis et satis dense reticulatis, lamina valde convexa, supra nitida, glabra et valde rugosa, subtus ad nervos albo-sericea, inter nervos laxa puberula ad glabrescens, margine valde revoluta, crasse coriacea.

Flores axillares solitarii, 4-meri, 1–2 mm longe pedunculati, pedunculo crasse angulato, sericeo-puberulo suffulti; calycis tubus supra ovario cca 1 mm longe prolongatus et attenuatus, albo-sericeus; lobi 4, lineari-spathulati, apicem versus leviter dilatati, 3–4 mm longi, utrinque puberuli. Bractae 2, lineares, 1–1.5 mm longae, ad capsulam supra basim adnatae, albo-sericeae. Capsula globosa loculicida, 2–3 mm in diam., lobulis calycinis persistentibus coronata, puberula ad glabriuscula. Semina reticulato-rugosa, 1–1.5 mm longa, margine exalata.

Holotypus: HAJB 35663; Cuba; Prov. Holguín (Oriente), Sierra de Nipe. **Isotypus:** HAC!

Obs.: Habitu et forma foliorum *Rondeletia plicatula* Urb. simillima sed ab ea foliis subtus glabrescentibus vel laxa puberulis, atque forma calycis fructusque clare differt. Forma et longitudine lobis calycinis atque foliis subtus glabrescentibus *Rondeletia paucinervis* Urb.

similis, sed ab ea, indumento atque reticulatione venarum discrepat. Pro characteris intermediis huius taxonis eum hybridogenum gaudere preferimus.

Arbusto; ramitas jóvenes pelositas, las adultas escabrositas. Estípulas triangulares, sericeas de 1 mm de largo. Hojas agrupadas hacia los extremos de las ramitas, que conservan las cicatrices estipulares. Pecíolos sericeos de 1–2 mm de largo. Hojas ovales, oblongo-ovales, suborbiculares u oblongas, de 3–9 mm de largo por 2–5 mm de ancho, glabras, brillantes y rugosas en el haz, el envés albo sericeo en los nervios laxamente pubérulos entre ellos. Lámina redondeada a obtusa en el ápice, a veces brevemente apiculada, estrechada hacia la base obtusa o aguda, mayormente muy convexa, rigidamente coriácea, el margen revoluto. Nervio medio hundido en el haz prominente en el envés, ensanchado hacia la base, los laterales en pares de 2–3 obsoletos en el haz, prominulos y reticulados en el envés.

Flores axilares 4-meras, solitarias. Pedunculo de 1–2 mm de largo, sericeo, anguloso. Hipantio globoso, de 1–2 mm de diámetro, albo-sericeo, tubo del cáliz prolongado y atenuado sobre el ovario, lóbulos del cáliz 4, linear-espatulados, 3–4 mm de largo, pelosos en ambas caras, bracteae 2, lineales, de 1 mm de largo, sericeos. Corola no vista. Cápsula globosa de 2–3 mm de diámetro, albo-sericea. Semillas de forma variada, reticuladas y algo rugosas, de 1–1.5 mm de largo.

Holotipo: HAJB 35663; Cuba; Prov. Holguín (Oriente); Sierra de Nipe.
Isotipo: HAC 30262 !

Por el aspecto general y forma de las hojas semeja mucho a la *Rondeletia plicatula* Urb. de la cual se distingue en tener lóbulos del cáliz lineal-espatulados y hojas glabrescentes en el envés.

La forma y tamaño de las flores y frutos son parecidos a los de la otra especie próxima, *Rondeletia paucinervis* Urb., pero de ella difiere en tener hojas pubérulas y reticulado-venosas en el envés. Por tener caracteres intermediarios entre las dos especies aquí mencionadas preferimos calificar este taxon como un híbrido.

***Rondeletia pachyphylla* Kr. et Urb. Symb. Ant. 1: 419. 1900**

(Syn.: *Rondeletia alaternoides* Griseb. non A. Rich.)

ssp. *pachyphylla*: (Fig. 8)

Folia ovata vel ovali-elliptica, utrinque obtusa vel basi truncata, 2–3 (–3.5) cm longa et 1–1.5 cm lata fere duplo longiora quam latiora, margine anguste vel late recurva, plusminus convexa, nervis lateralibus supra nullis, subtus obsoletissimis.

Hojas aovadas o aovado-elípticas, obtusas en ambos extremos o truncadas en la base, de 2–3 (–3.5) cm de largo y 1–1.5 cm de ancho, eca dos veces mas largas que anchas, el margen estrechamente o anchamente recurva, el limbo convexo, nervios laterales nulos en el haz, muy evanidos en el envés.

Holotypus: WRIGHT 2689 GOET; isotypi: BM, GH, HAC.

Specimina examinata: Prov. Oriente: Sierra de Nipe: Paso Estancia to the pinales, SHAFER 1717, May 1–2. 1909, NY, US; idem, SHAFER 1769 May 1. 1909, BM, NY; — Río Piloto, EKMAN 3392 Nov. 5. 1914. S; EKMAN 9048 Febr. 18. 1918. S, US; — Río Canapú, EKMAN 4771. Febr. 25. 1915. S; Río Canapú, Cayo Rey; LÓPEZ F. 1864; Febr. 18. 1955. HAC, HAJB, US; — Farallones del Cayo Rey, CARABIA 3545. Apr. 15. 1940. GH, HAC, NY; — Río



Fig. 8. Authentic specimen of *Rondeletia pachyphylla* Kr. et Urb. (MARIE-VICTORIN 22064)

del Medio near Woodfred, MORTON 3263, Oct. 18. 1941. MORTON et ACUÑA, HAC, K, NY, US; — Arroyo Casimba, alt. 700 m. Jul. 27. 1940. LEÓN 19234. HAC, GH, NY, US; — Rio Naranjo, MARIE-VICTORIN 22301. March 14–18. 1944. M.-VICTORIN et CLEMENTE, HAC, MT; idem, LEÓN 19289, Jul. 28. 1940. LEÓN et ALAIN, HAC, NY; — Rio Piedra, 500 m.; EKMAN 1768. Jul. 3. 1914. S; — Rio Barigua, EKMAN 16058, Dec. 24. 1922. BM, GH, S; — Rio Bayate, EKMAN 1981, Jul. 13. 1914. S; — Rocky Arroyos near Woodfred, 400–500 m. SHAFER 3164, Dec. 13. 1909. GH, NY, S; — idem, MARIE-VICTORIN 22077, March, 14–18. 1944. M.-VICTORIN et CLEMENTE, GH, HAC, MT; — Pinelands near Woodfred, HOWARD 6126, July, 1941. GH, NY, MT; idem, LEÓN 19284, HAC, NY; idem, LEÓN 19941, Apr. 7. 1941. LEÓN, CLEMENTE et ALAIN, GH, HAC, NY; — idem, SHAFER 3443, Jan. 5. 1910. NY; — idem, LEÓN 19174, Jul. 26. 1940. LEÓN et ALAIN, HAC, NY; — idem, LEÓN 19824, Apr. 6. 1941. LEÓN, M.-VICTORIN, CLEMENTE et ALAIN, GH, HAC; — Pié de la Loma Mensura, 725 m. alt. EKMAN 5767/b, May 16. 1915. NY, S.

ssp. myrtilloides Fernandez et Borhidi ssp. nova (Fig. 9)

Folia oblongo-elliptica vel oblongo-obovata, basi longe cuneata, 1–2 (–2,7) cm longa et 0,4–0,8 (–1) cm lata, 2,5–3-plo longiora quam latiora, margine plerumque plana, non convexa, nervis lateralibus saepe utrinque prominulis.



Fig. 9. Holotype of *Rondeletia pachyphylla* Kr. et Urb. ssp. *myrtilloides* Fernandez et Borhidi in HAC; CLEMENTE 6217

Holotypus: CLEMENTE 6217 HAC; Prov. Holguin (Oriente), Moa: Cerro de Cananova, arroyito del puente Colorado. Leg.: CLEMENTE, NESTOR et CRISÓGONE, Aug. 1948. Isotypus: NY.

Specimina examinata: Oriente: Moa: Río Yagrumaje, ACUÑA 12766, Apr. 14. 1945. HAC, US; idem, CLEMENTE 3636, May 19. 1944. HAC, MT; — idem, WEBSTER 3790, Jul. 17. 1951. MICH; — Río Moa, ACUÑA 12765, Apr. 10. 1945. HAC, US; — idem, LEÓN 20192, Jul. 22. 1941. LEÓN, CLEMENTE et HOWARD, GH, HAC, NY; — Cerro de Miraflores, Cananova, LEÓN 21153, Jul. 1942. GH, HAC, NY; — Río Cayuguán, MARIE-VICTORIN 21768, 27–31. May, 1943. HAC, MT, US; — idem, ALAIN 851, Jul. 1949. ALAIN et CLEMENTE, GH, HAC, NY, US; — Playa de la Vaca, MARIE-VICTORIN 21453, Apr. 16–23. 1943. M.-VICTORIN, ALAIN et CLEMENTE, HAC, MT, NY, US; — idem, BORHIDI, MUÑIZ et VAZQUEZ, March, 25. 1970. BP, HAC; — idem, BORHIDI, CAPOTE et OVIEDO Sept. 13. 1974. BP, HAC; — Arroyo Ocuje, MARIE-VICTORIN 21550, 16–23. Apr. 1943. M.-VICTORIN et ALAIN, HAC, MT, US; — Centeno, MARIE-VICTORIN 21472, Apr. 16–23. 1943. M.-VICTORIN, CLEMENTE et ALAIN, HAC, MT; — 15 km SW of Moa mill, HOWARD 5884, Jul. 1941. A; — idem, HOWARD 5982, GH, MT, U; — idem, LEÓN 20192, 20247, col.: LEÓN, CLEMENTE et HOWARD GH, HAC, NY; — El Coco, LEÓN 22684, Aug. 1945. LEÓN, CLEMENTE et ALAIN, HAC, NY; — Arroyo Jicotea, LEÓN



Fig. 10. Holotype of *Rondeletia papayoensis* Fernandez et Borhidi in S. EKMANN 9306

20145, Jul. 22. 1941. LEÓN, CLEMENTE et HOWARD, HAC, NY; — Campo San Benito, SHAFER 4088, Febr. 24. 1910. NY; — Campo La Gloria, SHAFER 8258, Dec. 24–30. 1910. NY;

Sierra del Cristal: Rio Miguel, ALAIN 5910, Apr. 2–7. 1955. ACUÑA, ALAIN et LÓPEZ F. HAC, NY;

Baracoa: Peña Prieta, Toa, ALAIN 3185, Jul. 22. 1953. GH, HAC, NY; — Gorge of Yumuri River, SHAFER 7816, NY, US; — idem, SHAFER 7831, Dec. 7–9. 1910. NY; — idem, LEÓN 18368, Jul. 1938. HAC, NY; — idem, LEÓN 17236, Aug. 1939. LEÓN et MARIE-VICTORIN, GH, HAC, NY; — 17 km S of Sabanilla, Via Azul, ALAIN 5147, 9081, Jan. 14. 1956. ALAIN et MORTON, HAC, NY, US; — Banks of Jauco River, LEÓN 11715, Jul. 17–Aug. 4. 1924. GH, HAC, NY, US. — Abra del Rio Yumuri, ALAIN 7658, 16. Jan. 1960. ALAIN, ACUÑA et RAMOS, HAC.

***Rondeletia papayoensis* Fernandez et Borhidi sp. nova (Fig. 10)**

Frutex; ramuli teretes, retrorso-pilosi, veteriores scabrosi. Stipulae oblongo-triangularis, longe subulatae, 4–6 mm longae, sericeae vel glabrescentes, reflexi et decidui. Petiolum 4–5 mm longum, hirsutum. Folia oblonga vel elliptico-oblonga leviter inaequilatera, 1,5–3,6 cm longa et 0,4–1,3 cm lata, apice obtusa vel acuta, brevissime apiculata, basi longe cuneata,

lamina supra pilosa, subtus albo-tomentosa, chartaceae vel subcoriacea; nervo medio supra conspicuo, subtus prominenti, lateralibus utroque latere 3-4, leviter impressis vel solummodo conspicuis, subtus prominentibus, valde curvatis versus apicem, strigillosis.

Inflorescentiae axillares, 1,5-2 mm longe pedunculatae capitatae, 3-5-florae, hirsutae. Bractae 2, triangulares vel lanceolatae 1-2 mm longae, sericeae. Calycis tubus subglobosus, 1-1,5 mm in diametro, hirsutus, lobi plerumque 4 rariter 5, triangulares vel deltoidei, basi usque dimidium connati, superne 1 mm longi, liberi, utrinque sericei, apice obtusi vel acutiusculi, in fructu reflexi. Tubus corollae 4-5 mm longus, pilis albis retrorso-hirsutus, lobi 4, suborbiculares, extus albo-hirsuti, 1-1,5 mm longi. Stylus brevissime puberulus. Capsula globosa, 2-3 mm in diametro, tomentosa, lobis calycinis coronata.

Obs.: *Rondeletia combsioidi* Fernandez et Borhidi affinis, que a specie nostra ramulis hornotinis 4-angulosis, pilis sursum directis antrorse hirsutis, foliis majoribus utrinque glabrescentibus vel glabris, nervibus lateralibus supra impressis subtus dense reticulatis differt. Altera species affinis *Rondeletia combsii* Greenm. characteribus aequalibus atque lobis calycinis triangulari-lanceolatis, 2-3 mm longis apice acutis statim distinguitur.

Ramitas cilindricas, retrorso-pelosas cuando jóvenes, las adultas escabrositas. Estípulas oblongo-trianguulares, largamente subuladas, de 4-6 mm de largo, sericeas a glabrescentes, reflejas y caedizas. Pecíolos hirsutos, de 4-5 mm de largo; hojas oblongas o elíptico-oblongas de 1.5-3.6 cm de largo por 0.4-1.3 cm de ancho, obtusas a aguditas en el ápice o muy corto apiculadas, atenuadas en la base; el limbo cartáceo o subcoriáceo, algo inequilátero, el margen plano o muy poco revuelto, estrigiloso-peloso en el haz, albo-tomentoso y estrigiloso en los nervios del envés. Nervio medio marcado en el haz, no prominente, estrigiloso hasta cerca de la mitad de la hoja, los laterales cuando aparentes, hundidos. Nervios prominentes en el envés, los laterales de 3-4 pares fuertemente encorvadas hacia el ápice, con una reticulación densa y marcada.

Inflorescencias axilares, pedunculadas, acabezueladas 3-5-floras, hirsutas; pedunculos de 1.5-2 mm de largo; brácteas 2, triangulares o lanceoladas, de 1-2 mm de largo, sericeas. Tubo del cáliz subgloboso, de 1-1.5 mm de diámetro, hirsuto, lóbulos mayormente 4, a veces 5, triangulares o deltoideos cortos, connados en la base hasta la mitad, la parte libre de 1 mm de largo, sericeos en ambas caras, obtusos a aguditos en el ápice, reflejos por lo menos en el fruto. Tubo de la corola de 4-5 mm de largo, retrorso peloso con pelos blancos, lóbulos 4, suborbiculares de 1-1.5 mm de largo, redondeados en el ápice, albo-hirsuto por fuera. Estilo peloso. Cápsula globosa de 2-3 mm de diámetro, tomentosa, coronada por los lóbulos del cáliz.

Afin a *Rondeletia combsioides* Fernandez et Borhidi, que difiere de nuestra especie en tener ramitas antrorso-pelosas, hojas mas grandes glabrescentes a glabras en ambas caras. Otra especie cercana *Rondeletia combsii* Greenm. sobre las características aquí mencionadas difiere también en tener lóbulos del cáliz triangular-lanceolados, agudos, de 2-3 mm de largo.

Holotypus: EKMAN 9306 S! Cuba, Oriente; Papayo prope Sevilla, in collibus ad litus. 27. 6. 1918. Isotypus: NY.

***Rondeletia peninsularis* Fernandez et Borhidi sp. nova (Fig. 11)**

(Syn.: *Rondeletia camarioca* sensu Alain quoad plantae provinciae Orientalis Cubae, non Wr. in Griseb.)

Frutex; ramuli hornotini leviter angulosi, flavo-hirsuti pilis retrorsis obtecti, veteriores cilindricei, glabrescentes, cicatricibus stipularum transversaliter fissurati. Stipulae interpetiolares sericei, triangulari-subulati, 3-4 mm longi. Folia 3-4 mm longe petiolata, elliptica



Fig. 11. Holotype specimen of *Rondeletia peninsularis* Fernandez et Borhidi in S. EKMAN 16167

vel oblongo-elliptica vel oblanceolata, 0,9–2 cm longa et 0,4–0,8 cm lata, apice obtusa vel rotundata, rariter mucronulata, basi obtusa vel acutiuscula, nervo medio lateralibus utroque latere 2–4 supra impressis, subtus prominentibus et manifeste denseque reticulatis, lateralibus sub angulo acuto abeuntibus ante marginem conjunctis; lamina supra tomentosa, subtus valde tomentosa et ad nervos sericeo-pilosa, margine revoluta, crasse coriacea.

Inflorescentiae axillares, 4–6 mm longe pedunculatae, 3-florae, flavo-hirsutae. Bracteae 2, triangulares, 1–2 mm longae, liberae. Flores 4-meri, sessiles, tubus calycis globosus, hirsutus, 1–1,5 mm in diam. leviter costulatus, lobi orbiculares vel suborbiculares, 1–1,5 mm longi, apice rotundati, utrinque tomentosi; tubus corollae retrorse pilosus. Capsula globosa, 2–3 mm in diametro, loculicida, tomentosa.

Obs.: *Rondeletiae camariocae* Wr. in Sauv. (e provinciis Matanzas et Las Villas) affinis, quae a specie nostra ramulis hornotinis pilis sursum directis hirsutis, foliis obovatis basi plerumque acutis vel cuneatis, nervis lateralibus supra obsoletis, lobis calycinis ovatis, apice obtusis, inflorescentiis brevius pedunculatis differt.

Ramitas cilindricas, amarillo-hirsutas, las más jóvenes retrorso-pelosas, perdiendo la pelosidad con la edad, con corteza rugosa manteniendo marcada las cicatrices estipulares. Estípulas sericeas, triangular-subuladas de 3–4 mm de largo; las hojas agrupadas hacia los

extremos de las ramas, pecioladas con pecíolos de 3–4 mm de largo, hirsutos. Limbo de la hoja elíptico, oblongo-elíptico u oblanceolado de 0.9–2 cm de largo y 0.4–0.8 cm de ancho, tomentoso por el haz, muy tomentoso y estrigiloso-sericeo en los nervios del envés, margen revuelto a subrevuelto, coriáceo, grueso. Nerviación aparente y hundida por el haz, prominente en el envés, los nervios laterales de 2–4 pares salen en un ángulo agudo, encorvados hacia el ápice, llegando hasta el margen, reticulación marcada.

Inflorescencias axilares, amarillo-hirsutas, 3-floras; pedunculos de 4–6 mm de largo; brácteas 2, triangulares, libres de 1–2 mm de largo. Flores 4-meras, sésiles, tubo del cáliz subgloboso, algo acostillado, hirsuto, de 1–1.5 mm de diámetro, tubo de la corola retrorsopelosa. Cápsula globosa, de 2–3 mm de diámetro, loculicida, tomentosa.

Afin a *Rondeletia camarioca* Wr. in Sauv. de las provincias de Matanzas y Las Villas, que difiere de nuestra especie en tener ramitas antrorso-pelosas, hojas obovadas, atenuadas a agudas en la base, nervios laterales poco visibles en el haz, lóbulos del cáliz aovados, obtusos en el ápice, inflorescencias más brevemente pedunculadas.

Holotypus: EKMAN 16167 S! Cuba; Oriente; Cabo Cruz, S. of Niquero, in limestone terraces facing the sea. 16. 1. 1923. Isotypi: US, NY.

Conocida sólo de la colección típica. Endémica.

***Rondeletia subcanescens* Fernandez et Borhidi sp. nova (Fig. 12)**

Frutex; rami hornotini 4-anguli, sericei, veteriores teretes et glabrescentes; internodii 1.5–7 cm longi. Stipulae late triangulares, apiculatae usque subulatae, 1–3 mm longae sericeae. Folia usque ad 3 mm longe petiolata, elliptico-oblonga vel obovata, 0.7–1.9 cm longa et 0.5–1 cm lata, apice rotundata vel obtusa, rariter acutiuscula, basi attenuata, cuneata vel obtusa, supra rugulosa, glabra et nitida, subtus pilis sericeis et tomento arachnoideo albo-tomentosa vel canescentia, margine revoluta vel subrevoluta, coriacea.

Inflorescentiae axillares 1–3-florae, sericeo-pilosae. Flores 4-meri, usque ad 1 cm longe pedunculatae, flores breviter pedicellati. Bractee 2, triangulares, usque ad 1.5 mm longae, sericeae. Calycis tubus cum hypanthio globosus, leviter 4-angulosus, usque ad 2 mm longus, sericeus; lobi 4, triangulari-ovati, 1.5 mm longi, basi connati, apice rotundati vel obtusi, reflexi, albo-sericei. Corollae tubus 6–7 mm longus, extus retrorse pilosus, intus glaber, fauce anillo lamellari praeditus; lobi 4, orbicular-obovati, 3 mm longi et lati, extus strigilloso-pilosi, intus arachnoideo-tomentosi. Stamina 4, medio tubi corollini inserta, filamenta usque ad 1 mm longa. Antherae lineares, dorsifixae, subsessiles, 2 mm longae, fauce corollae insertae. Flores brevistylis tantum visi; stylus 2–2.5 mm longus, basi minute pilosulus, apice bilobulatus, lobi cca 1 mm longi, stigma lineare. Capsula globosa, 4–5 mm in diam., loculicide dehiscens. Semina forma varia usque ad 2–2.5 mm longa, reticulato-rugosa, fertilia tenuiter alata.

Holotypus: CLEMENTE 6900 HAC; Cuba; prov. Holguin (Oriente). Camino de Centeno, Cerro de Miraflores, Cananova, Moa; serpentine barrens. Col.: CLEMENTE, ALAIN et CRISÓGONE; July, 1949. Isotypus: ALAIN 999, NY.

Specimina examinata: CLEMENTE 7389 HAC; Cuba. Prov. Holguin (Oriente). Camino de Cananova a Moa. Manigua del 1^{er} arroyo (Saltadero); serpentine barrens. Col.: CLEMENTE, NESTOR et CRISÓGONE. August–October, 1950. GH, NY, US. — M. VICTORIN 21594 HAC, MON, US; Charrascal de Playa de Vaca; Col.: M. VICTORIN, CLEMENTE et ALAIN, 16–23. 4, 1943.



Fig. 12. Isotype specimen of *Rondeletia subcanescens* Fernandez et Borhidi in NY; ALAIN 999

Obs.: *Rondeletiae lomensi* Urb. (e Baracoa) affinis, quae a specie nostra ramulis retrorse strigilloso-pilosis, foliis duplo majoribus, subtus posterior glabrescentes, tubo corollino cca duplo longiore, lobis corollinis supra glabris clare differt.

Ramitas jóvenes cuadrangulares y sericeas, cilíndricas y glabrescentes con la edad. Entrenudos de 1.5–7 cm de largo. Estípulas anchamente triangulares, apiculadas a subuladas de 1–3 mm de largo, sericeas. Hojas oblongo-aovadas a elíptico-oblongas de 0.7–1.9 cm de largo por 0.5–1 cm de ancho, rugosas, glabras y brillantes por el haz, albo-sericeas y densamente tomentosas por el envés. Pecíolo de hasta 3 mm de largo. El ápice redondeado a obtuso, a veces agudito, estrechando hacia la base obtusa a agudita. El margen revuelto, el limbo rigidamente coriáceo. Nervio medio hundido en el haz, prominente en el envés y ensanchado hacia la base, los laterales de 2–3 pares inconspicuos en el haz, ligeramente hundidos o poco prominulos en el envés con una reticulación aparente.

Inflorescencias axilares, 1–3-floras, estrigiloso-sericeas. Flores 4-meras, pedunculos de hasta 1 cm de largo, pelosos, pedicelos muy cortos. Cáliz globoso, ligeramente 4-anguloso de hasta 2 mm de largo, sericeo; lóbulos 4, triangular-aovados de hasta 1.5 mm de largo, unidos

en la base, el ápice redondeado a obtuso, reflejos y albo-sericeos. Brácteas 2, triangulares, de hasta 1.5 mm de largo. Tubo de la corola 6–7 mm de largo, retrorso-pelosa por fuera, glabro por dentro, con un anillo de denticulos en la garganta, lóbulos 4, orbicular-obovados, de 3 mm de largo y ancho, estrigiloso por fuera, araneoso-pelosos por dentro. Estambres 4, insertos sobre la mitad del tubo, filamentos de hasta 1 mm de largo. Anteras lineales, dorsifijas, subsésiles de 2 mm de largo, insertas en la garganta del tubo de la corola. Estilo (en la flor brevistila) corto de 2–2.5 mm de largo, poco aplanado, diminutamente pelosito en la base, bilobulado arriba, lóbulos de 1 mm de largo, estigma lineal. Cápsula globosa, pelosa, de 4–5 mm de diámetro. Semillas de 2–2.5 mm de largo, algo rugosas y reticuladas, las fertiles aladas y apendiculadas en uno de sus extremos.

Holotipo: CLEMENTE 6900 HAC; Cuba; prov. Holguin (Oriente). Camino de Centeno, Cerro de Miraflores, Cananova, Moa, sobre serpentina. Col.: CLEMENTE, ALAIN y CRISÓGONE, Julio, 1949. **Isotipo:** ALAIN 999, NY.

Ejemplares examinados: CLEMENTE 7389 HAC; Cuba; prov. Holguin (Oriente). Camino de Cananova a Moa. Manigua del 1^{er} arroyo (Saltadero), sobre serpentina. Col.: CLEMENTE, NESTOR y CRISÓGONE, Agosto–Octubre, 1950. GH, NY, US. — M. VICTORIN 21594 HAC, MON, US; Charrascal de Playa de Vaca; Col.: M. VICTORIN, CLEMENTE et ALAIN, 16–23. 4. 1943.

Afin a *Rondeletia lomensis* Urb. que difiere de nuestra especie en tener ramitas retrorso estrigiloso-pelosas, hojas de 2–4 cm de largo, glabrescentes en el envés con la edad, tubo de la corola de 10–11 mm de largo, lóbulos de la corola glabros por dentro.

***Rondeletia toaensis* Fernandez et Borhidi sp. nova (Fig. 13)**

Frutex; rami hornotini subteretes, pilis erectis ferrugineis dense puberuli, annotini gibberosí. Stipulae interpetiolares 1–1.5 mm longae, late triangulares, apice rotundatae et in mucrone 0.5 mm longo terminatae, basi annuliformiter connatae. Folia subsessilia, usque ad 1 mm longe petiolata, suborbiculari vel late elliptica, antice rotundata vel obtusiuscula, basi breviter attenuata et in petiolum protracta, 6–10 mm longa et 5–7 mm lata, nervo medio supra impresso, lateralibus 2–3 sub angulo 70–80° abeuntibus, supra nullis, subtus planis vel, paullo impressis, dense reticulatis, margine recurva vel revoluta, brunneo-nigrescentia in sicco, supra valde plicata, manifeste convexa, subtus opaca, supra glabra, subtus ad nervos strigulosa, crasse coriacea.

Fructus tantum visus; in axillis foliorum solitarii, pedicelli crassi, 1 mm longi. Prophylla usque ad 1.5 mm longa, in tubum involucriformiter connata, quoad libera, lanceolata, 1 mm longa, acuta, stipellis binis late triangularibus duplo brevioribus interjectis. Capsula globosa, 3 mm longa, sericeo-pilosa; lobi 4, triangulari-lanceolati, erecti (non reflexi uti in *R. diplocalyx* Urb.), 3 mm longi.

Holotypus: ALAIN 3494; Cuba; prov. Guantánamo (Oriente). Serpentine barrens, Peña Prieta, La Magdalena, Toa. Alt.: 600 m.s.n.m. 30. July, 1953. HAC; isotypi: NY.

Obs.: Habitu et forma foliorum *Rondeletiae vacciniifoliae* Britt. affinis, quae a specie nostra, stipulae basi non connatae, foliis utrinque glabris, calycis lobis linearibus, apice acutis usque ad 4 mm longis clare differt. Altera species proxima, *R. diplocalyx* Urb. foliis majoribus, ovatis, apice apiculatis vel acutis, margine plerumque planis, limbo folii utrinque glabro, lobis calycinis reflexis simplice viso distinguitur.

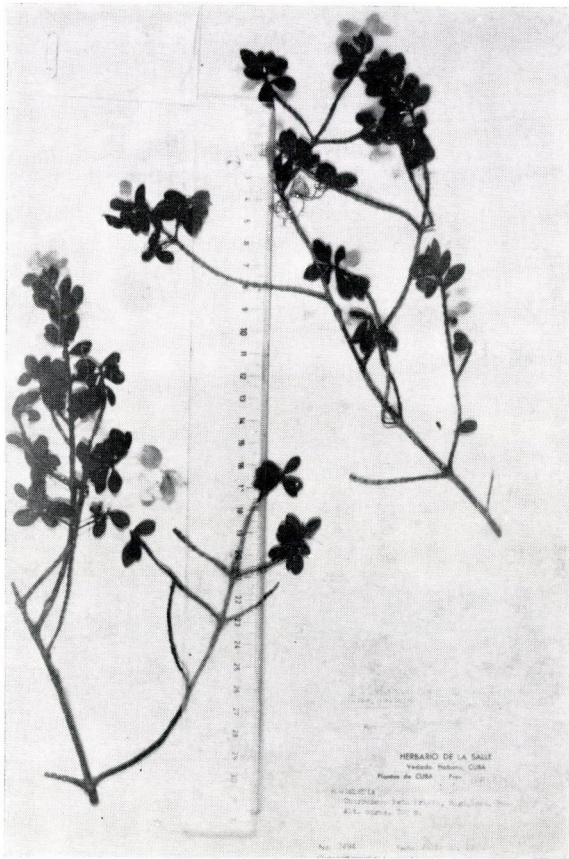


Fig. 13. Holotype specimen of *Rondeletia toaensis* Fernandez et Borhidi in HAC; ALAIN 3494

Arbusto; ramitas subcilíndricas, densamente pubérulas con pelos erguidos ferruginosos, las adultas verrugosas. Estípulas interpeciolares de 1–1.5 mm de largo, anchamente triangulares, redondeadas en el ápice con un mucron de 0.5 mm de largo, connadas en la base formando un anillo de 0.5–1 mm de largo. Hojas subsésiles con un pecíolo de hasta 1 mm de largo, suborbiculares o anchamente elípticas, redondeadas u obtusiusculas en el ápice, brevemente estrechadas en la base hacia el pecíolo, el limbo de 6–10 mm de largo y 5–7 mm de ancho; el nervio medio hundido en el haz, los laterales de 2–3 pares saliendo en un ángulo de 70–80°, nulos en el haz, aplandos o poco hundidos en el envés con un retículo denso; el margen recurvo o revoluto, el limbo bruno-negruzco en seco, muy plegado en el haz, convexo, mate en el envés, glabro en el haz, estrigiloso en los nervios del envés.

Flores no vistas. Frutos solitarios en las axilas, pedicelos gruesos, de 1 mm de largo. Prófilas de hasta 1.5 mm de largo, connadas en un tubo en forma de involucro, los lóbulos libres lanceolados de hasta 1 mm de largo, agudos con estípelas 2, anchamente triangulares, de mitad de largo, entre ellas. Cápsula globosa, de 3 mm de diámetro, sericeo-pelosa, lóbulos 4, triangular-lanceolados, erguidos (no reflejos como en la *Rondeletia diplocalyx* Urb.) de 3 mm de largo.

Holotipo: ALAIN 3494 HAC; Cuba; prov. Guantánamo. Serpentine barrens, Peña Prieta, La Magdalena, Toa. Alt.: 600 m.s.n.m. Col.: ALAIN, 30. 7. 1953. Isotipo: NY.

Afin a *Rondeletia vacciniifolia* Britt. que difiere de nuestra especie en tener estípulas connadas en la base, hojas glabras en ambas caras, lóbulos del cáliz lineares de 4 mm de largo. Otra especie próxima, *R. diplocalyx* Urb. difiere en tener hojas mas grandes aovadas, agudas y apiculadas, glabras en ambas caras, lóbulos del cáliz reflejos.

NEW TAXA IN THE *TILIA*-HERBARIUM OF JÁNOS WAGNER

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The author revised the valuable herbarium of the famous *Tilia*-specialist, János WAGNER and found 10 taxa new to science, 3 hybrids and 7 forms, partly denominated in the schedules but never published. This paper presents the correct descriptions of the mentioned taxa. They are: *Tilis* × *pseudolongirostris* Wagn. ex Vöröss hybr. nova, *T.* × *pseudopulchra* Wagn. ex Vöröss, hybr. nova, *T.* × *praelustris* Wagn. ex Vöröss habr. nova; *T. argentea* Desf. var. *colurnifolia* Borb. f. *subglobularis* Wagn. ex Vöröss f. nova, *T. grandifolia* Ehrh. var. *copiosa* Wagn. f. *praestabilis* Wagn. ex Vöröss, f. nova, *T. platyphyllos* Scop. var. *aenobarba* (Borb.) Jáv. f. *pseudo-trichogyna* Vöröss f. nova, *T. platyphyllos* Scop. var. *tenuifolia* (Host) Šimk. f. *sublaevigata* Wagn. ex Vöröss f. nova, et f. *grandibracteata* Vöröss f. nova; *T. caucasica* Rubr. var. *angulata* Rupr. f. *seiuntobracteata* Vöröss f. nova, *T. euchlora* C. Koch f. *pseudomultibracteata* Vöröss f. nova.

Introduction

In the *Tilia*-herbarium of J. WAGNER (see VÖRÖSS 1984) 525 taxa are represented and further 10 *Tilia*-taxa collected and separated by WAGNER. Some of them has also names given by him and some of their characteristic features are also mentioned in the schedules. The correct descriptions of these taxa, however, have not been published.

Descriptions of the new taxa

Tilia argentea Desf. var. *colurnifolia* Borb. f. *subglobularis* Wagn. ex Vöröss
f. nova

Sicut var. *colurnifolia* Borb., sed nuculis globosis, 7 mm in diametro.

Holotypus: Central Hungary: Vác-rátót; leg.: J. WAGNER, 9 September 1924. BP.

Tilia × *pseudolongirostris* Wagn. ex Vöröss **hybr. nova**
(*Tilia argentea* × *petiolaris*) Plate I, Fig. 1.

Sicut *Tilia* × *pulchra* Wagn., sed nuculis rostro 2 mm longo praeditis.

Holotypus: SW-Hungary; Comit. Somogy, Ságvári erdő; leg.: J. WAGNER, 19 August 1925. BP.

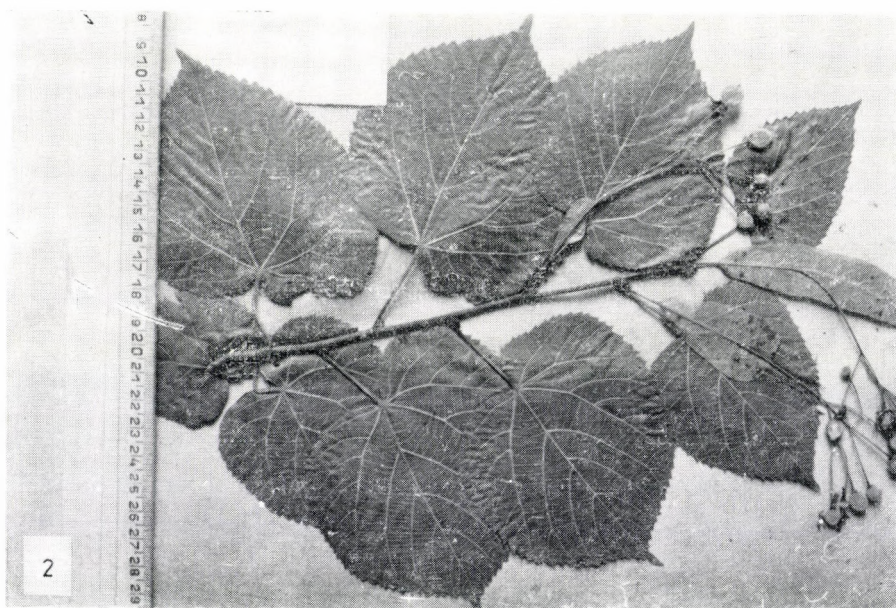
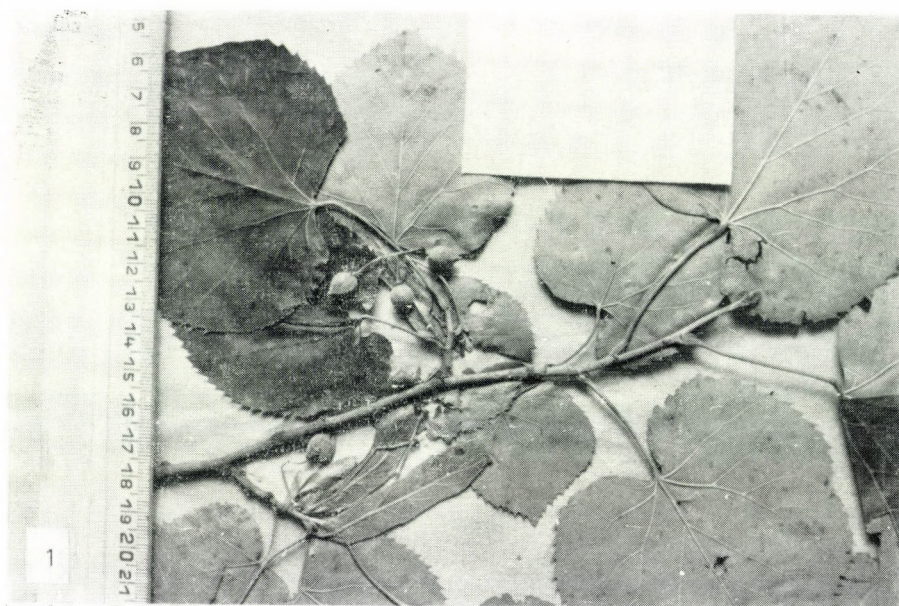


Plate I

1. *Tilia* × *pseudolongirostris* Wagn. ex Vöröss (Photo: Vöröss)
2. *Tilia grandifolia* Ehrh. var. *copiosa* Wagn. f. *praestabilis* Wagn. ex Vöröss (Photo: Vöröss)

Tilia* × *pseudopulchra* Wagn. ex Vöröss **hybr. nova*

(*Tilia argentea* var. *calvescens* × *petiolaris*)

Sicut *Tilia pulchra* Wagn., sed folia subtus minus tomentosa, viridiora quam apud *T. argentea* var. *calvescens*.

Holotypus: South-Hungary; Baja, near to the Serbian Church, cultivated; leg.: J. WAGNER, August 1930. BP.

***Tilia grandifolia* Ehrh. var. *copiosa* Wagn. f. **praestabilis** Wagn. ex Vöröss f. **nova**. Plate I, Fig. 2.**

Folia 8–10 cm longa et lata, infima eorum basi cordata, suprema oblique cordata vel oblique desecta, dentibus 2 mm longis, acuminatis, in foliis supremis dentibus acutis. Cyma 2–4 cm longior quam bractea; bractee usque ad 8 cm longae et ad 2 cm latae, rudimentales vel absentes, in petiolum decurrentes vel basi angustatae, petiolo nullo vel usque ad 2 cm longo.

Holotypus: Central Hungary; Comit. Pest: Nagykőrös, cultivated; leg.: J. WAGNER, 17 June 1926. BP.

***Tilia platyphyllos* Scop. var. *aenobarba* (Borb.) Jáv. f. **pseudo-trichogyna** Vöröss f. **nova**. Plate II, Fig. 3.**

Folia 3–5 cm longa, apice acuta, infima eorum basi cordata, cetera desecta. Bractee 5–6 cm longae, 10–12 mm latae, lanceolatae, petiolo nullo. Cymae bracteis 3–4 cm longiores. Stylus pilosus. Nuculae costis 8, emergentibus, 9 × 7 mm.

Holotypus: South Hungary; Szeged: Népkert (Public garden); leg.: J. WAGNER, 15 June 1932. BP.

***Tilia platyphyllos* Scop. var. *tenuifolia* (Host) Simk. f. **sublaevigata** Wagn. ex Vöröss f. **nova**. Plate II, Fig. 4.**

Sicut var. *tenuifolia* (Host) Simk., sed bractee 9–12 cm longae, 1.5–2 cm latae, lineares vel lanceolatae, infimae breviter petiolatae, supremae carum epetiolatae, nuculae leviter costulatae, parum tomentosae, globosae, 6 mm in diam., parum apiculatae.

Holotypus: West-Hungary; Comit. Veszprém, Pápa: Tanítóképző (Teacher's College); leg.: J. WAGNER, 3 October 1931.

Specimina examinata: West-Hungary; Comit. Sopron, Eszterháza (actually: Fertőd), leg.: J. WAGNER, 25 August 1933.

***Tilia platyphyllos* Scop. var. *tenuifolia* (Host) Simk. f. **grandibracteata** Vöröss f. **nova**. Plate III, Fig. 5.**

Sicut var. *tenuifolia* (Host) Simk., sed bractee 10 cm longae, 2 cm latae, nuculae 10 × 5 mm, rostro 1 mm longo.

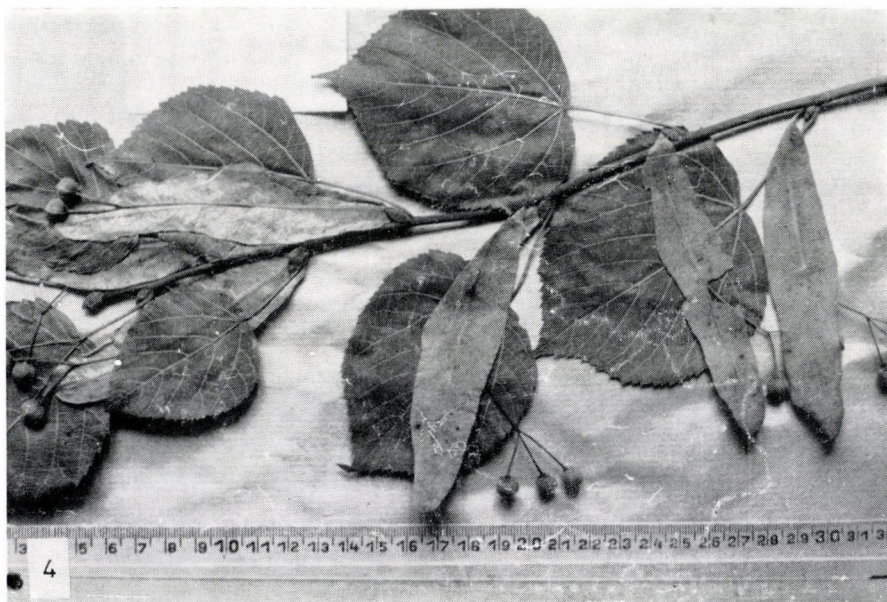


Plate II

3. *Tilia platyphyllos* Scop. var. *aenobarba* (Borb.) Jáv. f. *pseudo-trichogyna* Vöröss (Photo: VÖRÖSS)
4. *Tilia platyphyllos* Scop. var. *tenuifolia* (Host) Simk. f. *sublaevigata* Wagn. ex Vöröss (Photo: VÖRÖSS)

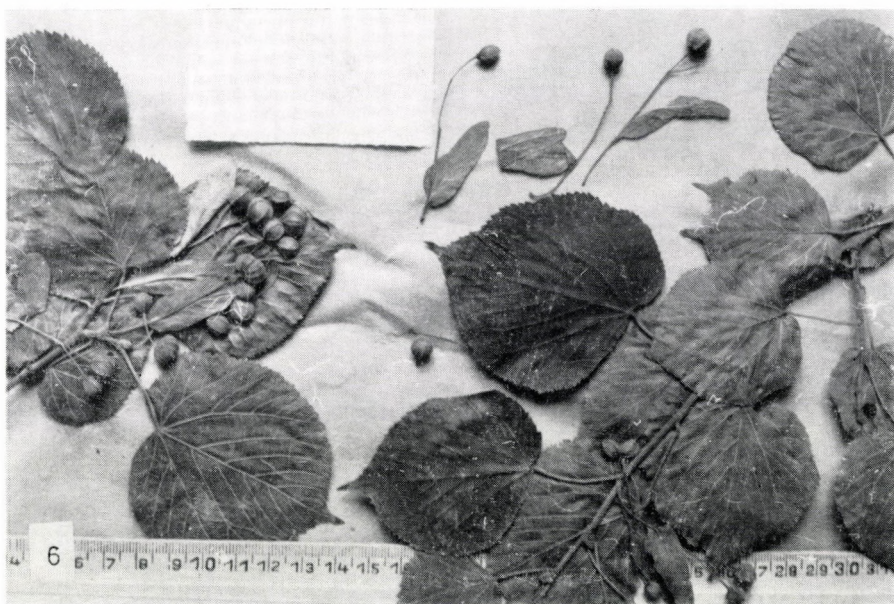
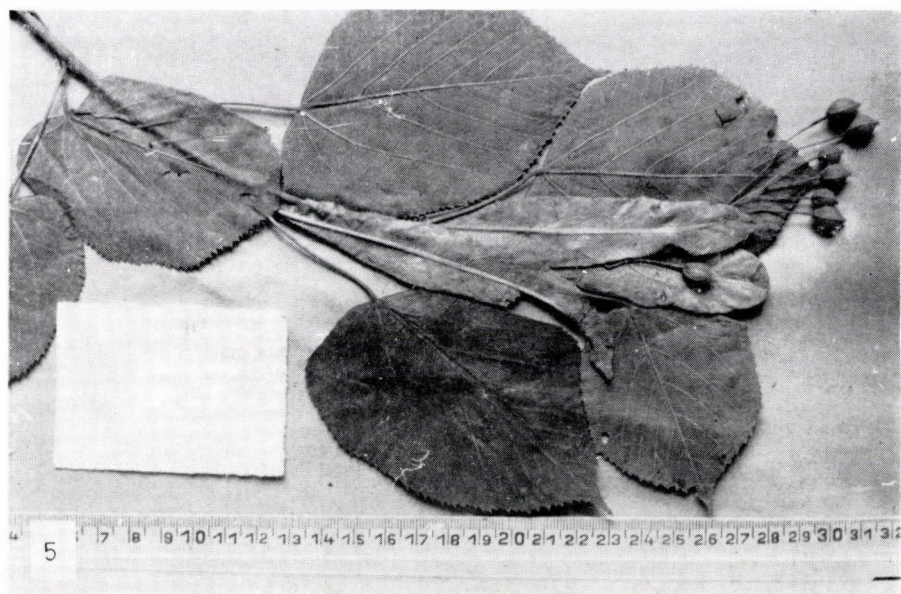


Plate III

5. *Tilia platyphyllos* Scop. var. *tenuifolia* (Host) Simk. f. *grandibracteata* Vöröss (Photo: Vöröss). 6. *Tilia caucasica* Rupr. var. *angulata* Rupr. f. *sejunctobracteata* Vöröss (Photo: Vöröss)

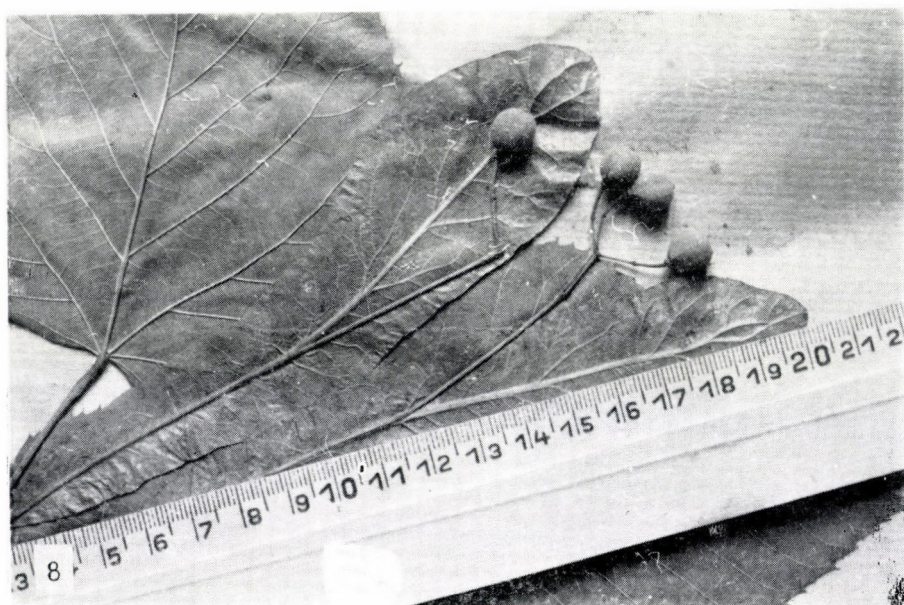


Plate IV

7. *Tilia euchlora* C. Koch f. *pseudo-multibracteata* Vöröss (Photo: Vöröss). 8. *Tilia* × *prae-lustris* Wagn. ex Vöröss (Photo: Vöröss)

Holotypus: Roumania, Comit. Arad; Mária-Radna; leg.: J. WAGNER, September 1906.

Tilia caucasica Rupr. var. *angulata* Rupr. f. *seiuntobracteata* Vöröss f. *nova*.
Plate III, Fig. 6.

Folia minora, lamina supra atroviridis, subtus pallide viridis, 5–6 cm longa, folia suprema basi desecta, cetera cordata, aequaliter serrata, dentibus aristatis. In angulis nervorum lateralium majorum barbulae brunneae oculo nudo conspicuae. Bractee 3–4 cm longae, 1 cm latae, \pm lineares, petiolo 2 cm longo. Cyma e basi bractee nascens vel libera. Nuculae parum deplanatae, parvae, 5–6 mm in diam., cortis valde emergentibus, saepe deformatae, costis ve 8–10 praeditae.

Holotypus: Hungary; Jobbágyi; leg.: J. WAGNER, 20 September 1926. BP.

Tilia euchlora C. Koch f. *pseudo-multibracteata* Vöröss f. *nova*. Plate IV, Fig. 7.

Sicut *Tilia euchlora* C. Koch, sed bractee saepe bracteola 25 mm longa et 4 mm lata praeditae.

Holotypus: West-Hungary; Comit. Sopron: Eszterháza (actually: Fertőd); leg.: J. WAGNER, 19 August 1931. BP.

Tilia \times *praelustris* Wagn. ex Vöröss **hybr. nova** (*Tilia* \times *Jakabiana* \times *T. petiolaris*); Plate IV, Fig. 8.

Folia usque ad 16 cm longa, 10 cm lata, basi oblique cordata, dentibus latis, apice breviter aristatis, aristis versus apicem folii curvatis, ad basim non barbata, sed in angulis nervorum lateralium brunneo-barbatula. Bractee epetiolatae, 16 cm longae, 4 cm latae. Nuculae globosae, 8 mm in diam., non profunde sulcatae, emergentibus 5 praeditae, apice parum excavatae, medio excavationis apiculo minuto suffultae.

Holotypus: South-Hungary; Szeged; leg.: J. WAGNER, 18 September 1924. BP.

Specimen examinatum: Ibidem, 3 July 1927., 18. September 1933. BP

The author wishes to dedicate this paper to the memory of Dr. János WAGNER.

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KARYOTYPE INVESTIGATIONS IN MONGOLIAN *AGROPYRON CRISTATUM* (L.) GAERTN. POPULATIONS

I. VARIATIONS OF PLOIDY LEVEL AND CHROMOSOME MEASUREMENTS

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In four *Agropyron cristatum* (L.) Gaertn. populations of Mongolia — a riverside-, two medium-height mountain- and a high mountain population — the number of somatic chromosomes, the relative length of chromosomes in metaphasis (C_{rel}) and the ratio of arms (a.r.) were determined on Feulgen-stained root-tip mitoses. The two medium-height mountain populations proved diploid ($2n = 14$), the riverside- and the high mountain population were found to be tetraploid ($2n = 28$). This differentiation by altitude can probably be traced back to the action of ecological genetic mechanisms and to flora history correlations. The chromosome measurements and the values of arm ratio varied differently in the four populations. The range of variation was widest in the riverside tetraploid population. In the variation of measurements and arm ratios the structural differences of chromosomes are likely to be manifested.

Introduction

The *Agropyron cristatum* (L.) Gaertn. is one of the most frequent grass species in the flora of the People's Republic of Mongolia. It is a stable member in many plant communities of the Mongolian forest steppe and dry steppe zones, but very often occurs extrazonally, on southern slopes in the mountain taiga zone, on the high-altitude plateaux and ridges, and in certain oases of the desert steppe and desert zone (DASHNJAM 1974; YUNATOV 1974). The crested wheatgrass has a very wide ecological amplitude, and probably that is the reason for its 10-70 per cent share from the total aboveground phytomass of pastures and grasslands in the dry steppe zone (BANNIKOVA and DYLLIS 1978; GORDEEVA et al. 1977; MIROSHNICHENKO 1967). Thus, as an excellent fodder plant it is of great economic importance.

According to YUNATOV (1950: 37) the gene centre of *Agropyron cristatum* is in Mongolia. His conclusion is based on the wide ecological amplitude and high morphological variability of populations found there. It is thus worth examining what karyotype variations occur in various stands of this grass species. Namely, it is remarkable that in this country under the extremely diversified environmental conditions, specific karyotype patterns may appear in the more or less isolated populations of often low individual number, as a result of natural selection, genetic drift, geographical or ecological isolation. Factors causing and maintaining such karyotype variations or even polymorphism may be in Mongolia the following physico-geographic conditions: the average 1580 m height above sea level; the cold continental climate; the highly diversified topographic forms and because of this very great microclimatic differences within small distances; the ecological isolation in the great number of larger or smaller closed basins, on the moving or semi-fixed sand-dunes, on the ridges and plateaux of inselbergs; the overlapping and mosaic pattern of vegetation zones; on the southern slopes the absence of vegetation characteristic of certain altitudes (e.g. the direct contact of the taiga- and dry steppe vegetation); in general, the increased fragility of the arid ecosystems.

According to our knowledge, apart from a publication on chromosome number (HANELT 1973) and an earlier one of ours (OJUNSUREN 1978) cytological data on Mongolian *Agropyron* have not been published so far. On the basis of the above we began studying the karyotype pattern in *Agropyron cristatum* populations of various sites with the view of obtaining new data and disclosing new cyto-ecological correlations, not to mention the practical importance. We did so in the hope that this work will serve as a model for studies on numerous karyologically unknown species of the Mongolian flora.

In this paper chromosome number and chromosome measurement data obtained with the classic Feulgen staining method are presented.

It should be noted that in some places of Mongolia *Agropyron pectinatum* (L.) Beauv. — probably introduced — also occurs (GRUBOV 1982), but TZVELEV (1976) considers it a subspecies of *A. cristatum*. Owing to the high degree and mutually overlapping polymorphism of the two Mongolian subspecies (*A. cristatum* ssp. *cristatum* and ssp. *pectinatum*) the taxonomic classification of populations studied by us is neglected and — following the opinion of TZVELEV — both subspecies are regarded as belonging to the taxon of *A. cristatum* (L.) Gaertn.

Material and method

In the summer of 1979 and 1980 we collected samples of ripe crested wheatgrass spikes at many sites of the forest steppe-, dry steppe- and desert steppe zones of the People's Republic of Mongolia. The samples were varying in size but each of them contained spikes of at least 100 plants per population. Present paper gives the results of examining four Mongolian samples. They were collected in the following places:

"S" — *Songino*, a steep, rocky southern slope of a high place above the right bank of the river Tola, some 20 km west of Ulan-Bator, at a height of about 1350–1450 m above sea level, some 50–150 m above the river-side willows, a rocky grassland containing desert steppe elements;

"L" — *rocky altitudes beside Lun*, about 130 km west of Ulan-Bator, 1100–1300 m above sea level, with a dry steppe vegetation;

"IB" — *the Ikh-Bogd Mountain* in the Gobi-Altay, some 580 km south-west of Ulan-Bator, 2800–3000 m above sea level, a ridge of rock-glacier with a vegetation of mountain steppe character;

"Sha" — *in the vicinity of Shaamar*, about 250 km north of Ulan-Bator, on sandy hills beside the wide left-hand flood plain of the river Orkhon, in the clearings and skirts of a thin *Pinus silvestris* L. forest, at a height of about 700 m.

The sites of origin of these four population samples are indicated in an outlined vegetation map of Mongolia (Fig. 1). The distance of the northernmost locality (Sha) from the southernmost one (IB) is 720 air kilometres or so, the distance in height above sea level some 2200 m. The annual amount of precipitation hardly exceeds 300 mm in Shaamar and 100 mm in the Ikh-Bogd Mountain.

For the purpose of comparison two samples of Hungarian *A. cristatum* ssp. *pectinatum* populations were also determined for chromosome number. One of them was collected on sandy hills near Dunavarsány, the other from a tumulus in the neighbourhood of Ohat.

The caryopses removed from the spikes and cleared of the awns were germinated in Petri-dishes on filter paper wetted with tap-water, in a thermostat of 22 °C, for about 48 hours, then placed in a 0.01–0.1 percent colchicine solution and further germinated for 4 hours. The roots, then 5–6 mm in length, were pretreated in a 0.002 mole oxychinoline solution at 13–15 °C for 3 hours, then fixed after ÖSTERGREN and HENEEN (1962) and GILYAROVSKAYA (1973) in a solution of the following composition:

| | |
|-------------------|-------|
| methanol | 60 ml |
| chloroform | 30 ml |
| distilled water | 20 ml |
| picric acid | 1 g |
| 2,4-dinitrophenol | 1 g |
| mercuric chloride | 1 g |

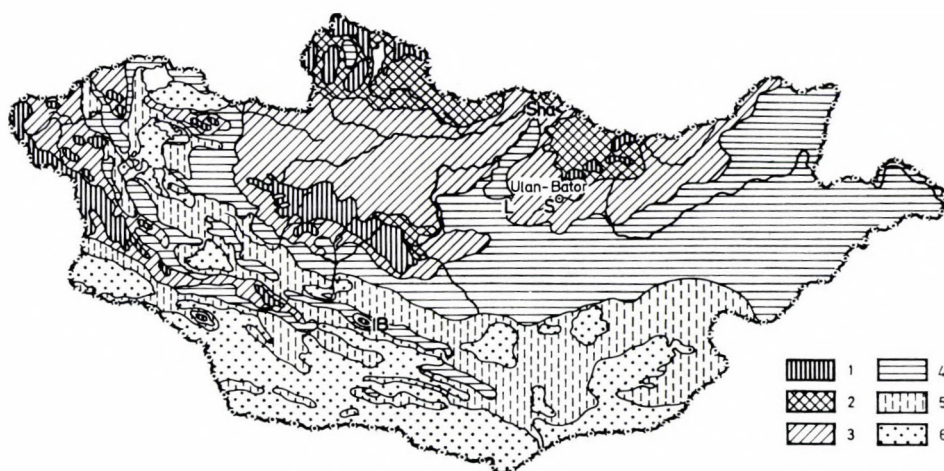


Fig. 1. Sites of the *Agropyron cristatum* (L.) Gaertn. populations studied on the phytogeographical map of Mongolia. — Populations: Sha — Shaamar; L — Lun; S — Songino; IB — Ikh-Bogd. — Vegetation zones: 1 — high mountain; 2 — taiga forest; 3 — forest steppe and meadow; 4 — dry steppe; 5 — desert steppe; 6 — desert

Fixing was carried out at room temperature for 12–18 hours, then the roots left in the fixer were stored in a refrigerator at $+3$ – $+5$ °C. Staining was carried out according to Feulgen: hydrolysis in 1 N hydrochloric acid at 60 °C for 8 minutes, then in cold hydrochloric acid for further 3 minutes; staining in Schiff's reagent at room temperature for 3 hours. After staining the roots were softened in an enzyme solution containing 4 percent pectinase and 2 percent cellulase, at 37 °C for 3–4 hours, then the root-tips were placed in aqueous chloral hydrate solution saturated cold on slides, covered with glass and crushed by pressing.

The preparations were examined by a Leitz Ortholux microscope, and the good metaphases photographed through a Zeiss MF adapter onto an ORWO NP-15 or NP-20, 6×6 cm film. Of the good negatives copies of 3000 total enlargement were made. It was on them that we carried out the measuring of chromosomes.

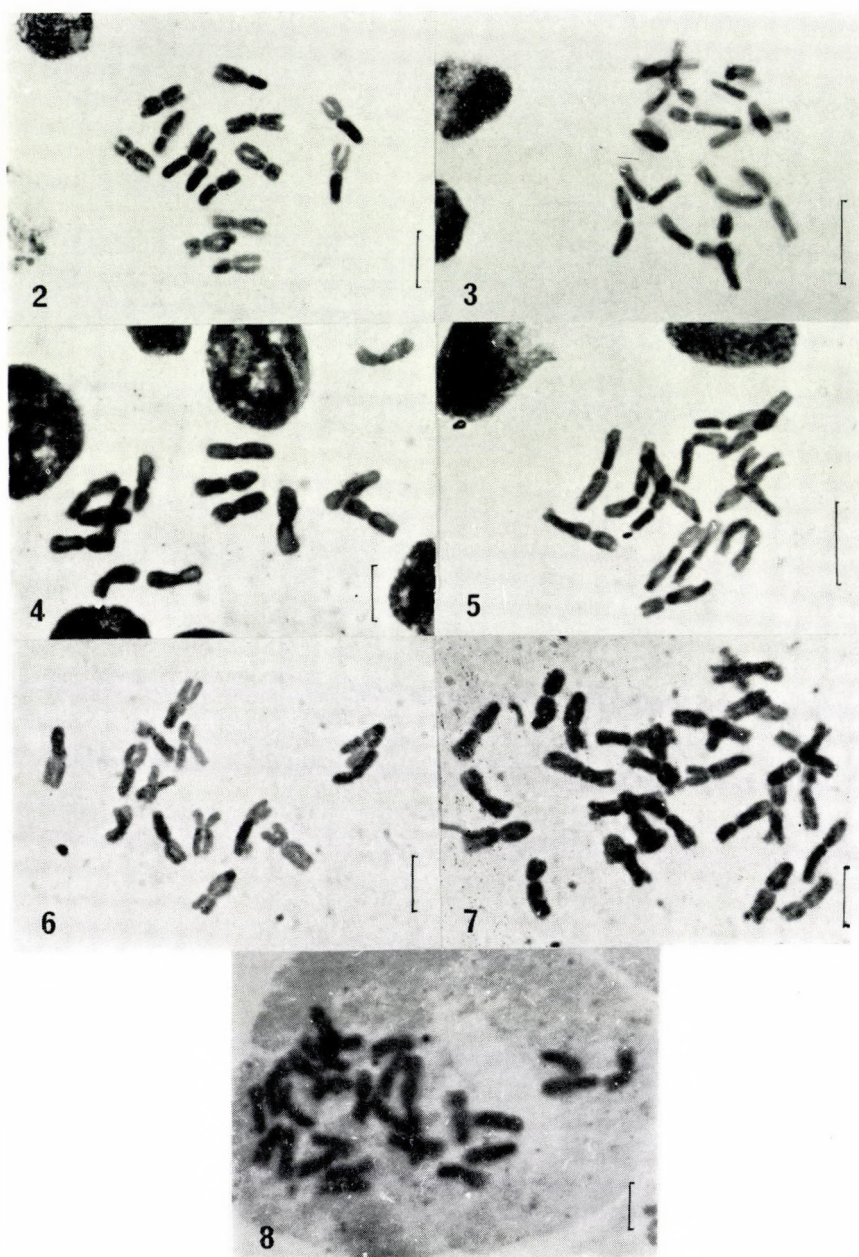
The number of somatic chromosomes was determined in 45–50 preparations of each population sample, on the microphoto enlargements of 10 preparations the chromosomes and their arms were measured, the total length of the somatic chromosome complement and the relative length of each chromosome (C_{rel}) within, as well as the arm ratios (a.r.) were determined. From these data averages for each population and limit values of confidence ($\pm h$) corresponding to the level of $P = 5\%$ were calculated (Sváb 1981). The chromosomes were arranged in a line on the basis of the arm's length ratios, and according to the terminology of LEVAN, FREDGA and SANDBERG (1964) placed either in the metacentric (M; a.r. ≤ 1.5) or in the submetacentric (S; a.r. > 1.5) class.

Results and discussion

1. Ploidy level

On the basis of the samples examined the "S" and "L" populations proved diploid ($2n = 14$) while the "IB" and "Sha" populations tetraploid ($2n = 28$). The two populations from Hungary are also diploid. The metaphase patterns characteristic of the two cytotypes are shown by microphotos (Figs 2–8).

Literary data concerning the geographical distribution of the diploid and tetraploid cytotypes of crested wheatgrass are relatively few (AVDULOV 1931; SOKOLOVSKAYA and



Figs 2–8. Characteristic chromosome complements of *Agropyron cristatum* (L.) Gaertn. populations in somatic metaphasis; Feulgen staining; the length of the line drawn on the right is 5 μ m. Populations: 2 — Songino; 3 — Songino with a satellite chromosome pair; 4 — Lun; 5 — Dunavarsány; 6 — Ohat; 7 — Ikh-Bogd; 8 — Shaamar

STRELKOVA 1948; SARKAR 1956; SCHULZ-SCHAEFFER and JURASITS 1962). Besides there are data on the existence of hexaploid stands too (ARARATYAN 1938; DEWEY and ASAY 1975).

It is known that in debates related with the distribution of polyploid plant species and cytotypes and with their geobotanical, flora and vegetation history role the disclosure of evolutionary genetic and ecological genetic correlations has promoted in many cases the clarification of details, though rules of general validity have not been found (HAGERUP 1932; LÖVE and LÖVE 1949; Soó 1947; LÖVE 1953; REESE 1958; FAVARGER 1967; STEIN 1970; WAGNER 1970; VIDA 1976).

In our material one of the tetraploid populations lives in the Gobi-Altay, at an altitude of 2800–3000 m, the other one on sandy hills beside the river Orkhon at about 700 m above sea level, but in a similarly severe microclimate. The two medium-height mountain populations, on the other hand proved to be diploid. According to DEWEY and ASAY (1975) in the Elbrus Mountain, Iran, the hexaploid cytotype of *Agropyron cristatum* is found at medium heights (about 1600 m), while at altitudes higher than that (above 2000 m) the cytotype of lower ploidy level (tetraploid) occurs.

The separation by altitude of the diploid and tetraploid populations in Mongolia is probably due to the action of ecological genetic mechanisms and to flora history correlations. Of the two tetraploid populations the one found in the river valley is supposed to be the younger. However, in our opinion these questions can be settled only after the examination of a larger number of population.

2. Chromosome measurements

The results of the biometric processing of chromosome measuring data are summarized in Tables 1 and 2. In Table 2 containing the data of tetraploid populations double C_{rel} values are given for the sake of easier comparison. The 4 idiograms representing the average karyotypes of the populations have been constructed on the basis of the data of tables (Figs 8 and 9).

Table 1

Relative lengths (C_{rel}) and arm ratios (a.r.) of *Agropyron cristatum* chromosome pairs in the two Mongolian diploid populations.
Average values and $P = 5\%$ confidence limit values

| Chromosome pairs | "S" (Songino) | | "L" (Lun) | |
|------------------|------------------|------------------|------------------|------------------|
| | C_{rel} | a.r. | C_{rel} | a.r. |
| M1 | 7.25 ± 0.164 | 1.06 ± 0.002 | 7.62 ± 0.349 | 1.05 ± 0.002 |
| M2 | 7.34 ± 0.383 | 1.11 ± 0.120 | 7.21 ± 0.305 | 1.13 ± 0.012 |
| M3 | 7.54 ± 0.246 | 1.21 ± 0.004 | 7.13 ± 0.076 | 1.21 ± 0.006 |
| M4 | 7.06 ± 0.186 | 1.34 ± 0.008 | 7.02 ± 0.165 | 1.28 ± 0.007 |
| M5 | 7.16 ± 0.239 | 1.43 ± 0.009 | 7.10 ± 0.380 | 1.40 ± 0.023 |
| S1 | 6.80 ± 0.088 | 1.54 ± 0.320 | 6.72 ± 0.145 | 1.53 ± 0.024 |
| S2 | 6.74 ± 0.066 | 1.85 ± 0.025 | 7.25 ± 0.281 | 1.76 ± 0.020 |
| $\bar{x} \pm h$ | — | 1.36 ± 0.069 | — | 1.33 ± 0.091 |

Table 2

*Relative lengths (C_{rel}) and arm ratios (a.r.) of *Agropyron cristatum* chromosome pairs in the two Mongolian tetraploid populations.
Average values and $P = 5\%$ confidence limit values*

| Chromosome pairs | "IB" (Ikh-Bogd) | | "Sha" (Shamar) | |
|------------------|------------------|------------------|------------------|------------------|
| | C_{rel} | a.r. | C_{rel} | a.r. |
| M1 | 7.06 ± 0.047 | 1.00 ± 0.000 | 6.80 ± 0.610 | 1.00 ± 0.000 |
| m2 | 7.26 ± 0.092 | 1.06 ± 0.006 | 7.22 ± 0.470 | 1.11 ± 0.000 |
| M3 | 7.08 ± 0.646 | 1.12 ± 0.001 | 5.86 ± 0.284 | 1.14 ± 0.000 |
| M4 | 7.74 ± 0.076 | 1.16 ± 0.008 | 6.34 ± 2.972 | 1.20 ± 0.004 |
| M5 | 7.58 ± 0.098 | 1.21 ± 0.715 | 8.11 ± 0.066 | 1.20 ± 0.000 |
| M6 | 7.10 ± 0.145 | 1.26 ± 0.070 | 6.64 ± 0.000 | 1.25 ± 0.000 |
| M7 | 7.16 ± 0.715 | 1.32 ± 0.090 | 6.54 ± 1.568 | 1.39 ± 0.000 |
| M8 | 7.40 ± 1.122 | 1.37 ± 0.080 | 7.40 ± 2.910 | 1.40 ± 0.004 |
| M9 | 6.84 ± 0.276 | 1.43 ± 0.715 | 7.79 ± 4.015 | 1.41 ± 0.000 |
| M10 | 6.84 ± 0.112 | 1.48 ± 0.011 | 7.37 ± 0.000 | 1.50 ± 0.000 |
| S1 | 7.32 ± 0.800 | 1.55 ± 0.715 | 7.77 ± 0.029 | 1.59 ± 0.000 |
| S2 | 6.94 ± 0.105 | 1.65 ± 0.012 | 6.30 ± 1.776 | 1.62 ± 0.008 |
| S3 | 7.02 ± 0.715 | 1.77 ± 0.018 | 7.35 ± 0.190 | 1.77 ± 0.004 |
| S4 | 6.76 ± 0.121 | 1.96 ± 0.024 | 7.62 ± 0.968 | 2.11 ± 0.004 |
| $\bar{x} \pm h$ | — | 1.38 ± 0.166 | — | 1.41 ± 0.002 |

In both diploid populations the somatic chromosome complement consists of 5 metacentric (M1–M5) and two submetacentric (S1–S2) pairs. The extreme values of the relative length of chromosomes are nearly the same in the two populations. However, in the "S" population the M3 chromosome pair, while in the "L" population the M1 pair is the longest, and in the former the S1 while in the latter the S2 pair the shortest. The highest variation of relative length is shown by the Lun M4 pair ($h = \pm 0.615$). In the diploid karyotypes the relative lengths of two chromosome pairs of identical serial number (M3 and S2) are significantly different ($P = 5\%$). Between the two diploid karyotypes there is a slight, non-significant difference in average asymmetry.

In the tetraploid populations the somatic chromosome complement equally consists of 10 metacentric (M1–M10) and 4 submetacentric (S1–S4) pairs of chromosome. In the Shamar population the relative lengths of the chromosome pairs range between wider limits than in the Ikh-Bogd population. In the former the variability of the individual chromosome pairs is also higher than in the latter. The relative length of three chromosome pairs of identical serial number (the M3, M5 and M6 pairs) significantly ($P = 5\%$) differed in the two tetraploid populations. In the average asymmetry of chromosomes a slight, non-significant difference was found between the tetraploid populations.

On the basis of the ratios of chromosome measurements certain difference in karyotype can be pointed out even between the two diploid populations of Mongolia, but remarkable differences in the distribution of measure-

ments are found in the two tetraploid populations. In the variation of karyotype within and between the populations the structural variations of chromosomes are likely to be manifested. These variations are supposed to be the results of such structural rearrangements (translocation, inversion, duplication, deletion) which occur with varying frequency in the different *Agropyron cristatum* populations. This hypothesis is made well-founded by the fact that the satellite chromosome pair could be pointed out by Feulgen staining only in the Songino population (Fig. 4).

At present of the light microscope cytological methods the banding techniques are the most suitable for the demonstration of chromosome variations resulting from the structural rearrangements. On caryological observations on *Agropyron* made with this method we shall give account in a following paper.

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KARYOTYPE INVESTIGATIONS IN MONGOLIAN AGROPYRON CRISTATUM (L.) GAERTN. POPULATIONS

II. C-BAND PATTERNS

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In four Mongolian *Agropyron cristatum* (L.) Gaertn. populations — one riverside-, two medium height mountain- and one high mountain ones — we studied the intra- and interpopulation variation of the constitutive heterochromatin distribution pattern by Giemsa staining of root-tip mitoses. From the large number of the Giemsa C-banding variants we have drawn the conclusion that the populations examined are characterized by high karyotypic polymorphism, partly parallel with the polymorphism of chromosome number and -measurement ratio we pointed out by Feulgen staining in the same populations (OJUNSUREN and JANKÓ 1985).

In the riverside- and high mountain tetraploid populations the number of C-bands of the somatic chromosome complement is significantly more than twice the band number characteristic of the diploid medium height mountain populations, and in the high mountain population it is larger than in the riverside one. The relative width of band is smaller in the tetraploids than in the diploids.

The correlation we found between the polymorphism of the C-banding pattern of Mongolian *Agropyron cristatum* populations and the height above sea level of the growing size agrees with what VOSA (1976) observed in populations of *Allium pulchellum* and KENTON (1976) in those of Mexican *Gibasis* species. From our data we may draw the conclusion that the polymorphism of heterochromatin distribution in the *Agropyron cristatum* populations — just like the separation of diploid and tetraploid populations by differences in height above sea level and microclimate, as reported in our previous publication — can be traced back to the action of evolution genetic and ecologic-genetic mechanisms and to flora history and vegetation history relations.

Introduction

Since the beginning of the seventies the chromosome band staining methods that made the reliable identification of chromosomes on the basis of the pattern of heterochromatin distribution possible in the human and animal cytology have been successfully used in the karyological and cytogenetic investigations of plants too (VOSA and MARCHI 1972, SARMA and NATARAJAN 1973, HADLACZKY 1975). Attention turned also to the polymorphism of heterochromatin localization that could be easier and more exactly examined with the help of band staining than with the conventional chromosome staining methods.

In investigations of this kind the heterochromatin polymorphism of the plant karyotype was found to be demonstrable not only in closely related species and infraspecific taxa (GREIL-HUBER 1978, LINDE-LAURSEN 1978a, LINDE-LAURSEN et al. 1980, THOMAS 1981), but in various populations of the same species too (FILION 1974, MARKS and SCHWEITZER 1974, BENTZER and LANDSTRÖM 1975, SINGH and RÖBBELEN 1975, WEIMARCK 1975, ABRAMOVA 1978, KENTON 1978). Differences in banding pattern were found e.g. between inbred lines and varieties of rye (TIKHONOVICH 1975).

The analysis of the distribution pattern of heterochromatin has been introduced in the evolution genetic investigations as well (GILL and KIMBER 1974a, b, HADLACZKY and

BELEA 1975, GILL 1981). In the karyotypes of interspecific hybrids and additive lines the origin of the different chromosomes could be determined after the banding pattern of heterochromatin (MERKER 1975, GUSTAFSON, EVANS and JOSIFEK 1976, LINDE-LAURSEN 1978a, b).

The number of plant species examined for karyotype by band staining is today over hundred (MEHRA and FOGLE 1982). To our knowledge in *Agropyron* Gaertn. and in the closely related genera of *Elytrigia* Desv. and *Elymus* L. analyses of this kind have not been performed so far.

In our previous publication (OJUNSUREN and JANKÓ 1985) we gave account of chromosome number differences and variations in chromosome size relations observed in Mongolian *Agropyron cristatum* (L.) Gaertn. populations. In this paper we wish to present the results of studies on karyotype variation by means of Giemsa band staining.

Material and method

For the investigations we used those Mongolian spike samples the origin of which was described in some detail in our above mentioned publication. Two of the samples (S — Songino and L — Lun) represented each a diploid population, while each of the samples IB — Ikh-Bogd and Sha — Shaamar a tetraploid population.

The heterochromatin localization was studied by the Giemsa staining of chromosomes after the description of HADLACZKY (1975). The localization of the constitutive heterochromatin (C-bands) in the chromosomes was studied in the dividing root-tip cells of at least 50 seedlings per population. We determined the place and number of C-bands in each chromosome of the somatic chromosome complement, the widths of the bands, the lengths of the chromosomes and of their arms, then from these data calculated the percentage proportion of the total band width to the total length of chromosomes (B%), and established the total number of C-bands in the somatic chromosome complement.

Results

In the dividing cells of the apical meristem of seedlings taken from the samples of *Agropyron cristatum* populations the distribution pattern of the constitutive heterochromatin (C-bands) in the chromosomes in metaphasis could be made visible by Giemsa staining. The Giemsa stained heterochromatin is generally localized in the telomeres of the *Agropyron cristatum* chromosomes (Fig. 1). Near the centromeres there are no C-bands, and intercalary bands rarely occur. By Giemsa staining chromosomes with satellites could even be pointed out; the satellite section of the nucleole-organizing chromosome was readily stained with Giemsa.

Telomeric bands can be observed not only in the metaphasis, but also in the prometaphasis, a less condensed state of the chromosomes, and are even detectable in the prophase (Fig. 2). By Giemsa staining the heterochromatin grains of nuclei in interphase can similarly be made visible.

Often two or three chromosomes in the chromosome complement show the same pattern of banding. In consequence of the polymorphism of heterochromatin distribution the staining pattern of a given pair of chromosomes is not always uniform in the different members of the population; in this case the chromosome pairs are difficult to identify on the basis of the pattern of banding. With a view to the reliability of identification we therefore determined the quantitative morphological features of the different chromosomes in the Giemsa stained preparations as well (arm length ratio, relative length).

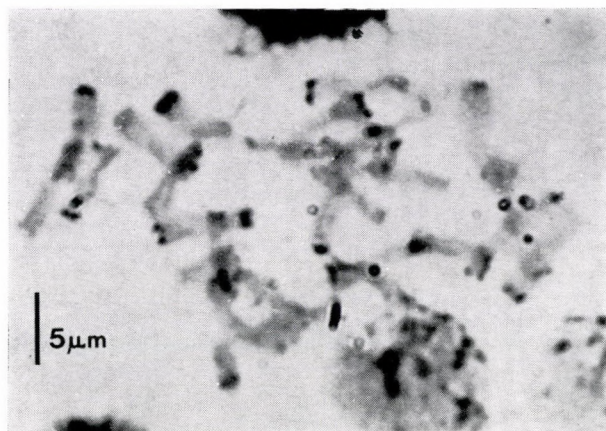


Fig. 1. Metaphase chromosomes of *Agropyron cristatum*, stained by Giemsa

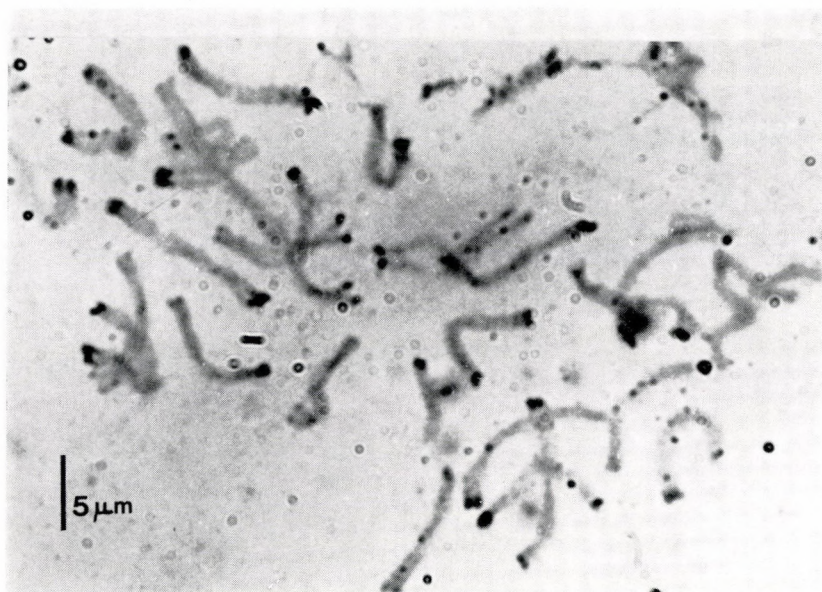


Fig. 2. Prometaphase chromosomes of *Agropyron cristatum*, stained by Giemsa

C-band pattern of diploid karyotypes

In the idiogram of Fig. 3 a typical heterochromatin distribution of the diploid chromosome complement is seen. The banding pattern of the 7 homologous pairs of the chromosome complement in both the Songino and the Lun population can be characterized as follows.

1. The M1, M2 and S2 chromosomes show telomeric C-bands on both arms.
2. In the M3, M4 and M5 chromosomes telomeric C-band is only seen on the shorter arm.

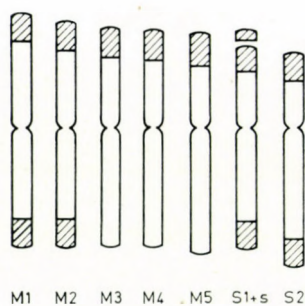


Fig. 3. Giemsa banding pattern of chromosomes, typical for our diploid population samples

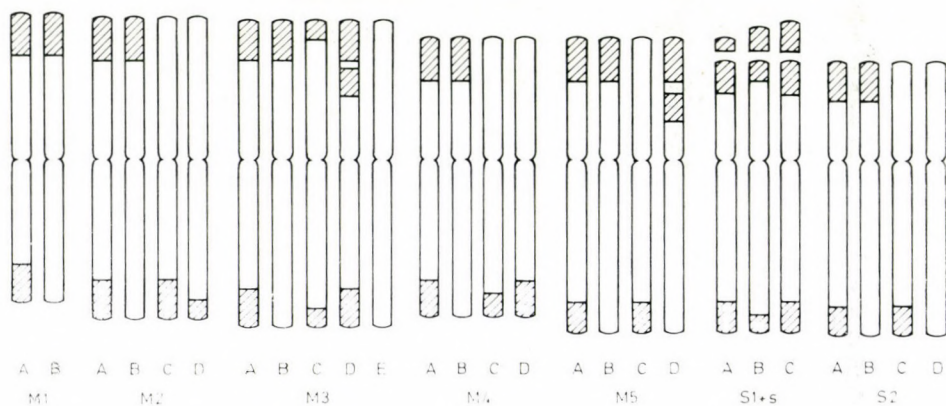


Fig. 4. Giemsa banding pattern variants in diploids, "Songino" population

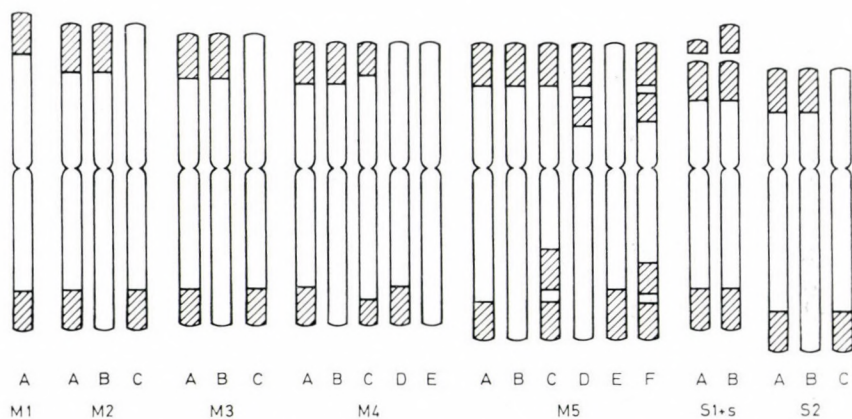


Fig. 5. The same as Fig. 4, "Lun" population

3. The $S1 + s$ (satellite) chromosome displays telomeric heterochromatin banding on both arms, and even the nucleole-organizing section stains indicating its heterochromatin content.

This basic pattern of heterochromatin distribution could be noticed in many preparations of both diploid population samples. Nevertheless, a considerable number of patterns other than that were also encountered. The idiograms of banding variants observed by us in the Songino and Lun population are shown in Figs 4 and 5. Figures 6 and 7 illustrate the relative frequency of the variants.

As seen in the figures the polymorphism of the C-band is manifest with each chromosome pair. As regards the pattern of C-banding some three-quarters of the homologous

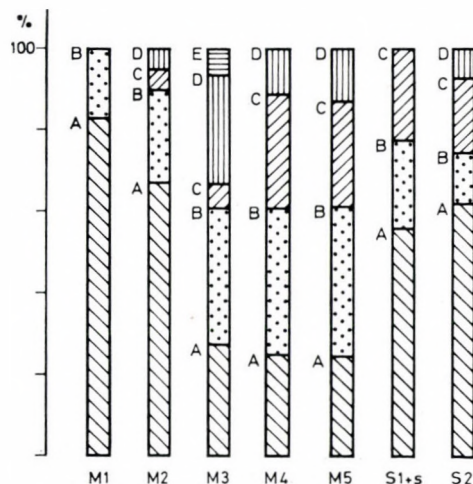


Fig. 6. Relative frequencies of Giemsa banding pattern variants in diploids, "Songino" population

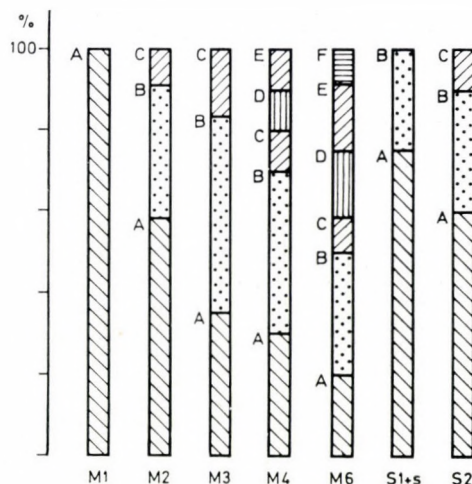


Fig. 7. The same as Fig. 6, "Lun" population

Table 1

*Number of C-bands and relative band width (B%)
in the karyotypes of diploid and tetraploid populations.
Mean values and $P = 5\%$ confidence limit values ($\bar{x} \pm h$)*

| | Number of C-bands | B% |
|-------------|----------------------|------------------|
| Diploids | | |
| Songino | 22.8 ± 1.38 | 16.86 ± 1.01 |
| Lun | 23.4 ± 0.90 | 17.51 ± 1.31 |
| Tetraploids | | |
| Ikh-Bogd | 52.3 ± 1.71 | 14.32 ± 1.14 |
| Shaamar | 51.0 ± 0.82 | 12.78 ± 1.23 |

pairs were homomorphous and one-quarter of them heteromorphous in each population. In the Songino population a total of 26, in the Lun population 23 banding variants were found. In spite of the diversity of the heterochromatin distribution, in most members of the populations 24 bands were contained in a chromosome complement, though there were plants with 20–23 bands. The ratio of total band width to total chromosome length (B%) ranged between 14.9 and 20.6 in the Songino and between 15.1 and 20.2 in the Lun population. Between the mean values of B% no significant difference was found (Table 1).

C-band pattern of tetraploid karyotypes

Figure 8 shows the idiogram of the pattern of heterochromatin distribution most characteristic of the tetraploid karyotype. Heterochromatin bands staining with Giemsa were found at both ends of most chromosomes. By Giemsa staining 2 chromosome pairs with satellites could be pointed out in each of the two tetraploid populations as well. In these chromosomes a telomeric band could also be seen beside the stained satellite.

In both tetraploid populations numerous banding variants different from the basic pattern were observed. In plants of the Ikh-Bogd population 49, while in those of the Shaamar population 53 banding variants could be differentiated. These variants are shown in Figs 9 and 10. In Figs 11 and 12 the relative frequency of the variants is seen. In most of the preparations obtained from the Ikh-Bogd population the total number of bands in a somatic chromosome complement was 54, and in a minor part of them ranged between 48 and 53.

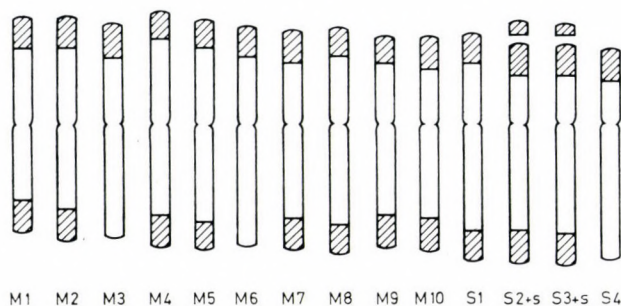


Fig. 8. Giemsa banding pattern of chromosomes, typical for our tetraploid population samples

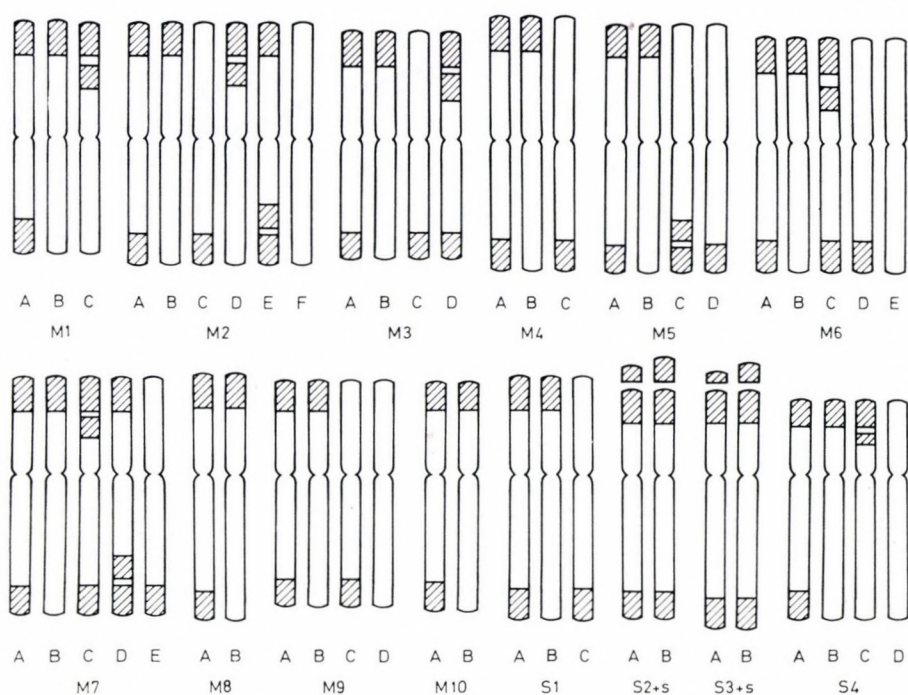


Fig. 9. Giemsa banding pattern variants in tetraploids, "Ikh-Bogd" population

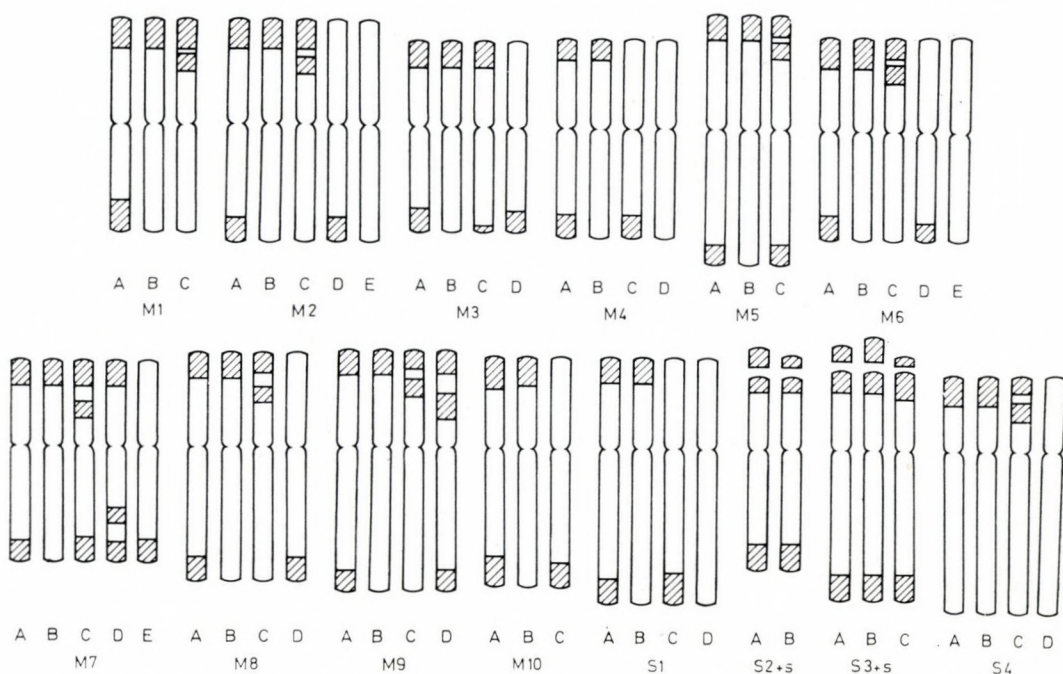


Fig. 10. The same as Fig. 9, "Shaamar" population

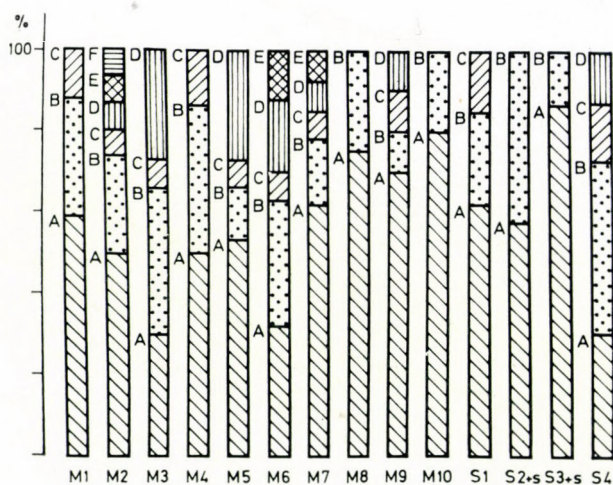


Fig. 11. Relative frequencies of Giemsa banding pattern variants in tetraploids, "Ikh-Bogd" population

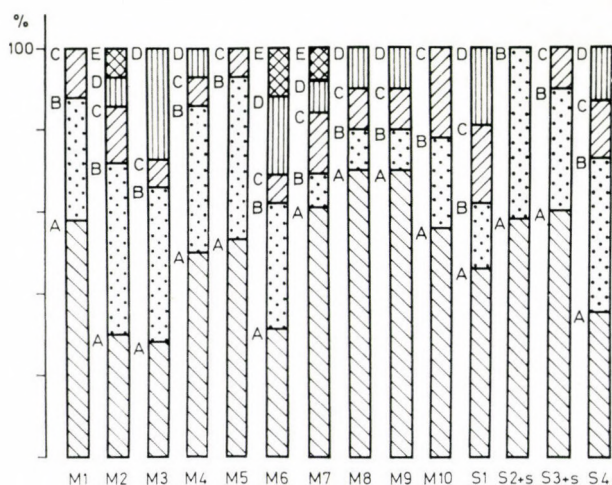


Fig. 12. The same as Fig. 11, "Shaamar" population

In half of the sample of the Shaamar population the total number of bands per chromosome complement was 52 and ranged between 49 and 51 in the rest. The B% values varied from 12.8 to 16.6 per cent in the Ikh-Bogd and from 10.4 to 15.1 per cent in the Shaamar population. The mean values of B% are much lower than in the diploid populations, while the total number of bands in the tetraploid chromosome complement is more than twice the number of bands in the diploid complement (Table 1).

Discussion

Series of analyses of C-banding — as referred to in the literary review — give account of the intraspecific polymorphism of heterochromatin. In some cases — e.g. in the species *Scilla sibirica* (VOSA 1973b), *Allium pulchellum* (VOSA 1976), *Anemone blanda* (MARKS 1974) — the polymorphism of C-banding was of such an extent that the material examined did not even contain two plants with the same pattern of C-banding.

The examination of the Mongolian *Agropyron cristatum* populations for heterochromatin distribution has revealed that these populations are also characterized by the polymorphism of heterochromatin, as a manifestation of the karyotype polymorphism. A large number of C-banding patterns could be differentiated. These variants were probably produced with the structural rearrangement of the chromosomes. However, in the karyotypic C-band polymorphism observed by us certain regularity could be established. In most plants of the two diploid populations of medium height mountains in the dry steppe zone of Mongolia the number of C-bands of the somatic karyotype was 24. The species obtained from the tetraploid Shaamar population living in the forest steppe zone, at a height of some 700 m above sea level mostly contained 52 C-bands in the somatic chromosome complement, while in plants from the Ikh-Bogd population living in the desert steppe zone, about 3000 m above sea level a karyotype with 54 bands was mostly found. Thus, the total number of bands in the tetraploids is in every case more than twice the band number of the diploid karyotypes, and among the Mongolian *Agropyron cristatum* populations the number of C-bands was highest in the somatic chromosome complement of the high mountain (Ikh-Bogd) plants. The same was observed by VOSA (1976): in three populations of *Allium pulchellum* the number of bands was in positive correlation with the height above sea level of habitats. From this fact VOSA concluded on the role of heterochromatin in adaptation. KENTON (1978) arrived at the same conclusion on the basis of the C-banding pattern in the Mexican populations of *Gibasis* species. Studying the karyotypes of *Allium flavum* populations of various habitats VOSA (1973a) noticed that the variability of heterochromatin distribution varied with the habitat, but each population was characterized by a certain type of polymorphism. From his observations (1973a, b) he drew the conclusion that the polymorphism of heterochromatin distribution is equal to the reflection of the populations' adaptation in the karyotype.

Our investigations contribute further data to support the above cited hypothesis of VOSA and KENTON. We may draw the conclusion that the heterochromatin polymorphism of the Mongolian *Agropyron cristatum* populations is related with the site conditions. It is the result of environmental effects, of actions of ecologico-genetic, evolution genetic mechanisms, of flora history

and vegetation history correlations, in the same way as the separation of diploid and tetraploid populations according to height above sea level and microclimatic differences discussed in our previous publication (OJUNSUREN and JANKÓ 1985).

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CYTOLOGICAL INVESTIGATION OF LONG-LASTING (SEVERAL GENERATIONS) HERBICIDE TREATMENT RESULTING SENSITIVITY CHANGES IN BARLEY

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Changes in the sensitivity of barley — effected by five years of herbicide treatment — have been investigated. The level of chromosomal aberrations in meiosis and mitosis was determined during five generations. It was demonstrated that in the first generations (M_1 — M_2) the frequency of chromosomal aberrations increased, than in the later generations (M_3 — M_5) returns to the control level. Regularly treated barley seeds show lower sensitivity in M_5 against repeated herbicide treatment than in previously untreated material.

Decrease of sensitivity is supposed to be the result of selection in the case of regular treatment.

Introduction

Sufficient data are available recently to support that some pesticides result mutations and chromosomal aberrations (EPSTEIN and LEGATOR 1974; CURINNY and PILINSKAYA 1976). Genetic effect of long-lasting use of a particular herbicide is not yet completely discovered. Though the number of well-known herbicides year by year increasing their assortment in agricultural practice is relatively narrow. Their use is therefore characterised by the following situation: the same herbicide is applied in a given agricultural district during several years.

After-effect investigation of herbicides is fairly contradictory. Some authors demonstrated that herbicide-treatment could have negative influence on progeny by damaging seed-corn qualities via changing some biochemical characteristics of seeds (MC CURDU et al. 1974; IVANENKO 1968). Other authors did not find any similar negative effect in barley (PETUNOVA 1971). FÜREDI et al. (1981) investigated cytological effects of some herbicides in pea after herbicide treatment of several generations. Application of herbicides during several years reduced the number of chromosomal aberrations.

In recent paper we report the results of cytological investigations (in meiosis and mitosis) from yearly treated with herbicide barley seed harvest of 1979–1983 years. We have determined the sensitivity of M_5 generation in the last year of treatment against the examined herbicides.

Material and methods

Two herbicides: Aniten D and Gabonil — currently used in Hungarian agricultural practice — were tested in our experiments for their mutagenic activity. Both of them were provided by the Plant Protection Research Institute of The Hungarian Academy of Sciences.

The trade names, international and chemical names and structural format of the tested herbicides and their effective substances (ingredients) are listed in Table 1. The concentrations of the given chemicals for the treatments were selected in proportion to the previously obtained LC_{50} values. Dry seeds were treated with freshly prepared aqueous

Table 1

Trade names, active ingredients and composition of the compounds tested

| Trade name | Active ingredient | Chemical name |
|------------|-------------------|--------------------------------------|
| Aniten D | 2,4-D + flurenol | 2,4-dichlorophenoxy-acetic acid |
| | | 9-hydroxyfluorene-9-carboxylic acid |
| Gabonil | MCPA + dicamba | 4-chloro-2-methyl-phenoxyacetic acid |
| | | 2-methoxy-3,6-dichloro-benzoic acid |

solutions of various concentrations at pH = 7.0 for 12 hours. Control seeds were soaked in distilled water. For investigations barley (*Hordeum vulgare*, 2n = 14) seeds of MV-43 strain were obtained from the Agricultural Research Institute of Hungarian Academy of Sciences, Martonvásár. The treatment was repeated during five (M₁—M₅) generations. The sowing was

Table 2

Frequency of meiotic cell with chromosome aberrations in treatments with

| Herbicides | Concentration (ppm) | Chromosome aberrations | | | | | | | |
|------------|---------------------|------------------------|-----------------|----------------------|------------|------------------|-----------------|----------------------|------------|
| | | M ₁ | | | | M ₂ | | | |
| | | Total no. plants | Total no. cells | Total no. abn. cells | Percentage | Total no. plants | Total no. cells | Total no. abn. cells | Percentage |
| Aniten D | 500 | 11 | 563 | 27 | 4.8 ± 0.9 | 9 | 521 | 18 | 3.5 ± 0.8 |
| | 1000 | 8 | 497 | 93 | 18.7 ± 1.7 | 7 | 386 | 13 | 3.4 ± 0.9 |
| Gabonil | 500 | 9 | 567 | 11 | 1.9 ± 0.6 | 11 | 448 | 6 | 1.3 ± 0.5 |
| | 1000 | 12 | 438 | 26 | 5.9 ± 1.1 | 7 | 521 | 15 | 2.9 ± 0.7 |
| Control | | 8 | 548 | 2 | 0.4 ± 0.3 | 9 | 454 | 2 | 0.4 ± 0.3 |

Table 3

Frequency of mitotic cell with chromosome aberrations in treatments with

| Herbicides | Concentration (ppm) | Chromosome aberrations | | | | | |
|------------|---------------------|------------------------|----------------------|------------|-----------------|----------------------|------------|
| | | M ₁ | | | M ₂ | | |
| | | Total no. cells | Total no. abn. cells | Percentage | Total no. cells | Total no. abn. cells | Percentage |
| Aniten D | 500 | 598 | 5 | 0.8 ± 0.4 | 401 | 3 | 0.7 ± 0.4 |
| | 1000 | 603 | 23 | 3.8 ± 0.8 | 362 | 11 | 3.1 ± 0.9 |
| Gabonil | 500 | 438 | 6 | 1.4 ± 0.5 | 421 | 8 | 0.9 ± 0.5 |
| | 1000 | 491 | 10 | 2.1 ± 0.7 | 389 | 8 | 2.1 ± 0.7 |
| Control | | 687 | 9 | 1.3 ± 0.4 | | | |

carried on outdoor experimental district. Each year and each repetition 200 seeds from harvest of the plot were treated in four repetitions. The size of sowing plots was 1 m². Tables 2 and 3 contain the summarised results of the four repetitions. After five years of re-sowing barley the herbicide sensitivity was determined on the experimental and control plots by the frequency of chromosomal aberrations in the root tip mitosis of the seedlings. For the analysis of meiotic chromosomal aberrations ears were fixed in 3 : 1 (w/w) ethanol-glacial acetic acid solution and stained with acetocarmine. The seeds of progeny were germinated on moist filter paper in Petri dishes and kept in 26 °C thermostat for 26–36 hours. After germination 3–5 mm long root tips were chosen for cytological analysis, carried on metaphase chromosomes of the root tip meristeme cells according to (PUSZTAI-GILYAROVSKAYA, 1973).

Results and discussion

The observed cytological effects of herbicides are presented in Table 2. During five years the meiotic analysis of yearly herbicide-treated progenies show that the found aberration frequencies in the first year (M_1) significantly exceeded the control ones. In the next year (M_2) the aberration frequency decreased at the treated plants. However frequency values exceeded the control level even in this case. In M_3 , M_4 , M_5 progenies the chromosomal

various herbicides and concentrations over five (M_1 – M_5) generation

| Chromosome aberrations | | | | | | | | | | | |
|------------------------|-----------------|----------------------|---------------|------------------|-----------------|----------------------|---------------|------------------|-----------------|----------------------|---------------|
| M_3 | | | | M_4 | | | | M_5 | | | |
| Total no. plants | Total no. cells | Total no. abn. cells | Percentage | Total no. plants | Total no. cells | Total no. abn. cells | Percentage | Total no. plants | Total no. cells | Total no. abn. cells | Percentage |
| 13 | 340 | 3 | 0.9 ± 0.5 | 14 | 638 | 3 | 0.5 ± 0.3 | 6 | 351 | — | — |
| 11 | 209 | 1 | 0.5 ± 0.5 | 9 | 401 | 5 | 1.2 ± 0.5 | 9 | 448 | 4 | 0.9 ± 0.4 |
| 9 | 501 | 3 | 0.6 ± 0.3 | 15 | 341 | 2 | 0.6 ± 0.4 | 5 | 327 | 2 | 0.6 ± 0.4 |
| 8 | 397 | 3 | 0.7 ± 0.4 | 7 | 428 | — | — | 11 | 398 | — | — |
| 10 | 438 | — | — | 6 | 521 | — | — | 9 | 498 | 2 | 0.4 ± 0.3 |

various herbicides and concentrations over five (M_1 – M_5) generation

| Chromosome aberrations | | | | | | | | |
|------------------------|----------------------|---------------|-----------------|----------------------|---------------|-----------------|----------------------|---------------|
| M_3 | | | M_4 | | | M_5 | | |
| Total no. cells | Total no. abn. cells | Percentage | Total no. cells | Total no. abn. cells | Percentage | Total no. cells | Total no. abn. cells | Percentage |
| 397 | 7 | 1.8 ± 0.6 | 405 | 7 | 1.7 ± 0.6 | 597 | — | — |
| 453 | 5 | 1.1 ± 0.5 | 501 | 15 | 3.1 ± 0.8 | 603 | 3 | 0.5 ± 0.3 |
| 387 | 4 | 1.1 ± 0.5 | 487 | 5 | 1.1 ± 0.4 | 687 | 6 | 0.9 ± 0.3 |
| 501 | 8 | 1.6 ± 0.6 | 397 | 6 | 1.5 ± 0.6 | 734 | 9 | 1.2 ± 0.4 |

aberration frequencies in meiosis practically remained on the control level. Necessary to note that Aniten D treatment in M_2 progeny greatly decreased the chromosomal aberration frequency in the case of 1000 ppm dose (in M_1 $18.7 \pm 1.7\%$ comparing to that of $3.4 \pm 0.9\%$ in M_2).

Chromosomal changes resulted by effect of herbicide treatment are of the following types: absent chromosome, chromosome fragment, bridges, chromosome stickiness, nuclear clumping and univalents.

From Table 3 (results of mitosis) it is obvious that in root tips of seedlings, originated from-treated with 1000 ppm Aniten D dose-seeds the frequency of chromosomal aberrations increased to $3.8 \pm 0.8\%$ while it was $1.1 \pm 0.4\%$ in the control. This effect was observed only at 1000 ppm dose of Aniten D. In M_2 progeny the mutagenic effect of herbicide treatment was not significant compared to the control, although some increase was observed in the case of Aniten D at 1000 ppm dose. The chromosomal aberration frequencies in M_3 , M_4 , M_5 are the same in the control and in the treated samples. Obtained data suggest that in first years of treatment the effect of herbicides manifest different to that of the latter years (after-effect). Frequency of herbicide-induced mutagenic changes in subsequent progenies decreased to the normal level. Experimental data suggest that the use of some herbicides during several years decrease the chromosomal aberrations in herbicide treated plants. In this case probably the mechanism of selection is involved, is not favorable for multiplication of individuals with chromosomal aberrations in plant population. Cells containing lethal-type chromosomal aberrations are sorting out and only cells carrying non-lethal ones get into the next generation. This is supported also by observation that regular treatments during several generation—especially in the case of 1000 ppm Aniten D treatment—result the decrease of plant stock.

Presumable in subsequent generations from the regularly herbicide-treated seeds the more resistant ones were selected out. Experiments were carried on to confirm this hypothesis in connection with herbicide-sensitivity of barley on regularly treated and control (untreated) seeds using one herbicide treatment.

Table 4
*Frequency of chromosome aberrations after five years
of treatments by herbicides in root tip meristems*

| Herbicides | Concentra- tions (ppm) | Control | | Treated | | Rate related to the control |
|------------|------------------------------|--------------------|---------------|--------------------|---------------|-----------------------------------|
| | | Total no. cells | Percentage | Total no. cells | Percentage | |
| Aniten D | 500 | 636 | 3.8 ± 0.7 | 930 | 2.7 ± 0.5 | 0.7 |
| | 1000 | 648 | 9.8 ± 1.2 | 728 | 4.3 ± 0.7 | 0.6 |
| Gabonil | 500 | 483 | 2.1 ± 0.6 | 683 | 2.8 ± 0.6 | 1.4 |
| | 1000 | 931 | 5.7 ± 0.7 | 870 | 4.1 ± 0.7 | 0.7 |

Table 4 represents changes in frequencies of chromosomal aberrations effected by new herbicide treatment in — earlier regularly treated and untreated — barley seeds. Accordingly the number of chromosomal changes in control seeds effected by the same dose of herbicide treatment was higher than in earlier several times treated seeds.

This data suggest to conclude that herbicide treatment — though differently — increase the frequency of chromosomal aberrations in mitosis and meiosis of M_1 — M_2 plants, while later progenies (M_3 — M_5) return to the level of the control. Earlier regularly treated barley seeds show lower sensitivity against repeated herbicide treatments.

Supposed the herbicide resistant forms survive in the population because herbicide resistance is determined not only by reparation mechanisms, but by morphological, physiological-biochemical (e.g. possibility to detoxicate) properties. One possibility to create stable forms in herbicide treated population by soring out (PINTHUS et al. 1972).

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CHARACTER RANKING FOR THE *SCILLA BIFOLIA* AGGREGATE IN HUNGARY BY THE APPLICATION OF SCAGA, A NEW TECHNIQUE OF CATEGORIZATION

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The method aims at preliminarily sieving dichotomous variables. The corresponding algorithm begins after having reduced all variables to dichotomous ones and also having eliminated variables neutral for discrimination. The program of our algorithm is based on a sequence of *assignment* and *releases* of individuals to or from categories. There is associated a "groups versus variable alternatives" type contingency table to each variable. Run begins with specifying the *maximum possible chi-square* of all contingency tables i.e., variables. Knowing now how strongly the *alternatives of the maximizing variable* are characteristic for one group or another the most likely corresponding set of individuals can be *assigned to a category*. If then the remaining (*waiting*) individuals yield, a variable for the same category when using the same algorithm this will be the second variable already. If, however, a different group emerges then those formerly categorized individuals of the opposite category are to be released which should have been categorized the other way by now. These individuals get back into the waiting group. The algorithm is implemented with distribution evaluations, significances, hit probabilities, counts for stages of categorization, stopping rule and description of decision course.

The above method has been applied by the authors as an up-to-date tool to the comparative taxonomical evaluation of Hungarian *Scilla bifolia* s.l. (Liliaceae) populations. In the framework of analyzing 23 characters of 260 individuals the rank order of those traits has been thoroughly investigated as an important information preparing more sophisticated distinctions among taxonomical values of the different groups.

In the present case study the SCAGA results were in good agreement with the hypothesized categories.

The importance and variability of characters describing the *Scilla bifolia* s.l. populations in Hungary are evaluated using a new method of categorization, SCAGA (Stepwise Category Assignment to Grouping Alternatives). The two-group version of the procedure is applied to the pairwise comparison of groups (i.e. localities) by finding the rank order of characters (variables). The characters are ranked according to their ability to discriminate between the alternative groups. Binary or dichotomized quantitative characters are used. In each step of the analysis a character and its states most typical of the groups are selected. Then, the individuals are assigned into two groups according to the dichotomy previously determined. In several steps of the analysis a new grouping of individuals results which may be contrasted with the actual geographical groupings. The rank order of characters obtained in several steps may stimulate new hypotheses and may confirm the results of other taxonomic and mathematical statistic studies.

Introduction

In the seventies a new approach to the identification of groups was introduced, instead of finding particular character combinations the rank order of characters showing their discriminatory power has been used (FELDMAN et al. 1969).

The advantage of this approach is obvious if we consider that characters may exhibit several, usually asymmetric, types of distribution and particular character states may be observed in one group only. Furthermore, it is important to simulate the hierarchic relationships among characters and some independence upon potential missing values in the data matrix is also often aimed at. In addition to these purposes, the objective of our studies was to develop a simple, problem-oriented method which is not intended to replace discriminant analysis (DA) but provide a technique for data screening prior to DA. This method finds the most important characters, i.e., those most selective for the majority of individuals. It is not a consequence, but a possibility, that the alternative state of a character proves selective for the rest of individuals.

The algorithm of SCAGA is different in many respects from the existing methods designed for similar purposes. A small subset of characters is selected which is as powerful in discriminating between groups as the full set. These characters are found by a ranking procedure. An advantage of this technique is that, in agreement with nature, in most cases assignment into one group is performed instead of dividing the set of individuals into two groups. Missing values often encountered in biological data matrices pose no computational problems, only the resulting lower χ^2 values will indicate this insufficiency. The method is also applicable to a few number of individuals. Also, it may render dispensable the use of more time consuming procedures for selecting discriminative variables. Most advantageous is the high certainty of finding essential features in the data. The method may be jointly used with other multivariate techniques, especially factor analysis.

Each step of categorization is based on the χ^2 values calculated from a four-way contingency table showing the distribution of each binary character over two groups of individuals. The stepwise and cumulative group membership probabilities are also used. The method and the subsequent evaluation of results aim at the logical groupings found after examining potentially relevant groupings and typical character choices. The main purpose is then to perform a preliminary categorization through character ranking which is compatible with the biological-diagnostic thought.

The new method was used in assessing the taxonomic importance of characters describing selected *Scilla bifolia* populations in Hungary (KERESZTY and SZILÁGYI 1984) in order to reduce the character set so that a subsequent DA may be efficiently used despite the relatively low number of individuals. The method was expected to confirm our hypotheses regarding the group structure of these populations.

The strategy of SCAGA

- The rank order of binary characters is determined. Multistate or quantitative characters are replaced by one or more dichotomous variables.
- In the first step ("assignment") the character for which χ^2 is maximum is selected. This character is given rank 1.
- The program informs us about the state of this variable and the locality to which the highest frequency value in the contingency table pertains.
- The individuals responsible for the maximum frequency cell of the contingency table are all assigned to a group for the time being. In an optimal situation this starting category will be identical with the maximum frequency category obtained at the end of the analysis.

— The individuals not assigned to the above category are put to a "waiting list". In the next step only these will be considered in search for the maximum χ^2 .

— If the individuals belong to the same group as above according to the new χ^2 , then the second character is the rank order is found. Otherwise, those ambiguously classified are also added to the waiting list.

— The analysis proceeds in a manner described above.

— If a group becomes empty, instead of χ^2 only the relative frequencies for the other groups will be considered in the ranking procedure. Nevertheless, the analysis is based on the maximum likelihood principle throughout.

— The final rank order of characters is given depending on the optional stopping rule.

The SCAGA program

1. Input data

A characters by individuals matrix which also indicates the group assignment of individuals.

2. Computations with the contingency table

Let A and B indicate the alternative groups. Let A* denote the statement that a given object is qualified as being a member of group A and, further, let B* denote that an object is assigned to group B, with assignments based on the character states observed. In each step of the ranking procedure a character is selected for which the expression

$$\chi^2 = \frac{(f_1 + f_4 - f_2 + f_3)(f_1 + f_2 + f_3 + f_4)}{(f_1 + f_2)(f_3 + f_4)(f_1 + f_3)(f_2 + f_4)}$$

is maximum. f_1, f_2, f_3 and f_4 are frequencies in the contingency table given below

| | | group | |
|----------|----|-------|-------|
| | | A | B |
| category | A* | f_1 | f_2 |
| | B* | f_3 | f_4 |

The magnitude of χ^2 , at a given number of individuals, shows how strong the relationship between group membership (A or B) and category membership (A* or B*) is and gives discrimination information on the distribution of the characteristic categories over the others. In case of binary variables the contingency table may be readily prepared, multistate or quantitative characters, however, have to be dichotomized using external or computed criteria.

In addition to the frequencies and χ^2 values, the output lists minima, maxima, means, standard deviations and the significance levels of χ^2 values.

3. Option for a detailed output

The choice is up to the user whether a 'short' or 'long' output list is printed by the program. In the long list the objects remained in each step are all printed and their distribution after the removal of each character is presented for every other character. In the short list distributions only for the actually found character are printed.

4. Indicators of efficiency

Potential re-assignments and their frequency, the number of objects on the waiting list and their proportion, and the number of correctly and erroneously categorized individuals per both group and total are printed in each step.

5. List of decisions

The series of tables in each step is followed by a summary of decisions. These are as follows: assignments or re-assignments made in order to find the rank order of characters and the global certainty of correct categorization.

Material

Twenty-six populations from thirteen regions in Hungary are represented in the sample. For detailed information on the populations and localities see the Fig. 1. The study material was collected since 1979. Then, all plants were grown under uniform conditions in the garden of the Research Institute for Botany, Vácrátót. In 1983 character states for 9 binary variables and measurements for 14 quantitative descriptors were recorded on 20 individuals from each population (Table 1). The complete data matrix contains 23 rows (variables) and 260 columns (individuals). The quantitative characters were measured at the time of full flowering, i.e., when all flowers in the inflorescence were open or almost open. To make these variables compatible with SCAGA each of them was dichotomized using various threshold values (see Table 1). The character set includes phenological and cytotaxonomical descriptors.

The data of the localities grouped according to XIII. regions are described below in following order: serial number of sample — name of nearest settlement — county (comitat) — geographical area and closer description or name of the locality — geobotanical area: floristic region and district — association — approximate altitude above sea level — date of collection — grid number on the flora map of Central Europe.

I. Upper Danube Valley

1. GYŐR; Győr com.; Püspökerdő, right after Pinyédi bridge; Eupannonicum-Arrabonicum; *Fraxino pannonicae-Ulmetum*; 100 m; 8. 5. 79; 8371. — **2.** ZSEJKEPUSZTA; Győr com.; Szigetköz, bank of the Danube; Eupannonicum-Arrabonicum; *Fraxino pannonicae-Ulmetum*; 100 m; 8. 5. 79; 8271. — **3.** VÁMOSSZABADI; Győr com.; Szigetköz, Határ-forest; Eupannonicum-Arrabonicum; *Quercu robori-Carpinetum*; 80 m; 8. 5. 79; 8272.

II. Central Danube Valley

4. KISOROSZI; Pest com.; Isle of Szentendre, bank of the Danube; Eupannonicum-Praematricum; *Fraxino pannonicae-Ulmetum*; 80 m; 4. 5. 79; 8180. — **5.** RÁCKEVE; Pest com.; Isle Angyali; Eupannonicum-Colocense; *Fraxino pannonicae-Ulmetum*; 80 m; 30. 4. 79; 8879. — **6.** MAKÁD; Pest com.; Isle Csepel, Makádi-forest; Eupannonicum-Colocense; *Fraxino pannonicae-Ulmetum*; 80 m; 30. 4. 79; 8979.

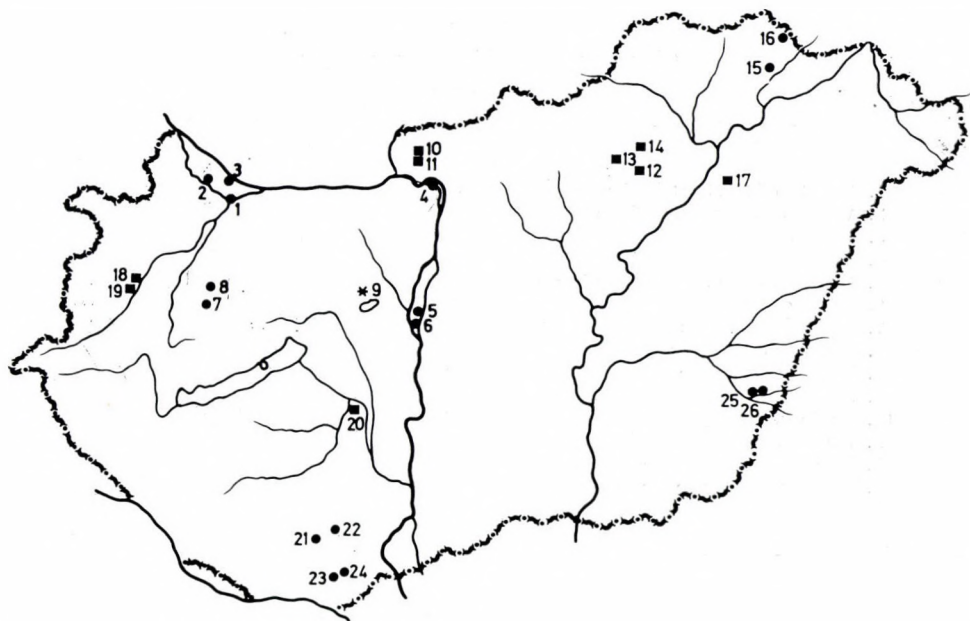


Fig. 1. Localities of the examined populations. Dots: diploids, squares: tetraploids, asterisks: hexaploids

IV. Velencei Mts.

9. NADAP; Fejér com.; Templom-Mt.; Bakonyicum-Veszprimense; *Aegopodio-Alnetum quercetosum*; 200 m; 10. 4. 79; 8777.

V. Börzsöny Mts.

10. NAGYBÖRZSÖNY; Pest com.; Nagyhideg-Mt., Mt.-top; *Matricum-Neogradense*; *Aconito-Fagetum*; 850 m; 27. 4. 79; 8079. — **11.** NAGYBÖRZSÖNY; Pest com.; Nagyhideg-Mt., K-Mt.-side; *Matricum-Neogradense*; *Aconito-Fagetum*; 800 m; 27. 4. 89; 8079.

VI. Bükk Mts.

12. CSERÉPFALU; Borsod com.; Valley of Hór stream; *Matricum-Borsodense*; *Melitti-Fagetum*; 250 m; 6. 5. 79; 8089. — **13.** FELSÓTÁRKÁNY; Heves com.; Ridge of Mt. Várhegy; *Matricum-Borsodense*; *Corno-Quercetum*; 600 m; 20. 4. 79; 8088. — **14.** RÉPÁSHUTA; Borsod com.; Kerek-Mt.; *Matricum-Borsodense*; *Aconito-Fagetum*; 650 m; 27. 4. 79; 8089.

VII. Zempléni Mts.

15. MAKKOSHOTYKA; Borsod com.; Valley of Völgypatak stream; *Matricum-Tokajense*; *Fago-Alnetum*; 350 m; 10. 5. 79; 7695. — **16.** FÜZÉRRADVÁNY; Borsod com.; Castle park of Vilypuszta; *Carpaticum-Cassovicum*; *Quercetum roboris* cult., in the place of a former *Fago-Alnetum*; 150 m; 8. 5. 80; 7595.

Table 1
Characters analyzed in the present study

| Character | Abbrev. | Threshold used |
|--------------------------------------|---------|--|
| 1. Length of the first leaf, mm | O | 100–150 |
| 2. Width of the first leaf, mm | W | 10–15 |
| 3. Ratio of O/W | L | 6–8–10–12 |
| 4. Length of stalk, mm | Z | 100–150 |
| 5. Length of the infloresc. axis, mm | A | 20–40–60 |
| 6. Ratio of Z/A | I | 1–2–3 |
| 7. Number of leaves | N | 2 |
| 8. Number of flowers | P | 2–4–5–8–10 |
| 9. Tepal length, mm | H | 6–8 |
| 10. Stamen length, mm | K | 6–8 |
| 11. Ratio of H/K | F | 1–2 |
| 12. Number of seeds per fruit | S | 10–15–20 |
| 13. Dry seed length, mm | M | 1.0–1.5 |
| 14. Chromosome number | C | 18–36–54 |
| Character states | | |
| 15. Elaiosome shape | E | 1: variable and grained 2: elongate |
| 16. Date of flowering | Y | 1: before 1. IV. — early 2: after 1. IV. — late |
| 17. Stem colour | G | 1: reddish 2: green |
| 18. Date of complete leaf withering | X | 1: before 25. V. — early 2: after 25. V. — late |
| 19. Root thickness | R | 1: thin 2: thick |
| 20. Bud colour | B | 1: green 2: blue |
| 21. Tepal colour | T | 1: white spotted 2: blue |
| 22. Colour of mature fruit | U | 1: green 2: reddish |
| 23. Colour of dry seed | D | 1: light ochre 2: dark brown |

VIII. Northern Hung. Plain

17. ÚJSZENTMARGITA; Hajdú com.; Nat. Park of Hortobágy, Margitai-forest; Eupannoni cum-Crisicum; *Galatello-Quercetum*; 100 m; 22. 5. 80; 8292.

IX. Western Transdanubian

18. IKERVÁR; Vas com.; Kemeneshát, Ikervári-forest; Praenoricum-Castriferreicum; *Quercrobori-Carpinetum*; 150 m; 12. 4. 79; 8767. — **19. IKERVÁR;** Vas com.; Kemeneshát, Iker-vári-meadow; Praenoricum-Castriferreicum; *Festuco-Alopecuretum* subcult.; 130 m; 12. 4. 79; 8767.

X. Tolnai Hill-country

20. PÁLFA; Tolna com.; Simontornyai-forest; Praeillyricum-Kaposense; *Quercrobori-Carpi-netum*; 200 m; 30. 3. 81; 9277.

XI. Mecsek Mts.

21. PÉCS; Baranya com.; Égervölgy valley; Praeillyricum-Sopianicum; *Helleboro odoro-Fagetum*; 200 m; 10. 4. 81; 9875. — **22.** PÉCS; Baranya com.; Tubes Mt.; Praeillyricum-Sopianicum; *Tilio argenteae-Fraxinetum*; 500 m; 11. 4. 80; 9875.

XII. Villányi Mts.

23. NAGYHARSÁNY; Baranya com.; Szársomlyó Mt., above the Szoborpark; Praeillyricum-Villányense; *Tilio argenteae-Fraxinetum*; 300 m; 10. 4. 79; 0176. — **24.** NAGYHARSÁNY; Baranya com.; Ördögölgy-valley; Praeillyricum-Villányense; *Asperulo taurinae-Carpinetum*; 160 m; 10. 4. 79; 0176.

XIII. Southern Hung. Plain

25. DOBOZ; Békés com.; Bank of the Kőrös, Fenyéresi-forest; Eupannonicum-Crisicum; *Quercu-Ulmetum*; 120 m; 29. 4. 79; 9293. — **25.** DOBOZ; Békés com.; Bank of the Kőrös, Vargahossza-forest; Eupannonicum-Crisicum; *Quercu-Ulmetum*; 120 m; 29. 4. 79; 9293.

Application of SCAGA

The populations were classified into four pairs so that four runs of SCAGA were performed. The contrasted pairs are as follow:

1. Populations from the hill-country and the mountains (HM) versus those from the plain (PL).
2. Populations from the Transdanubian mountains (TD) versus those from the northern mountains (NM).
3. Populations from the Danube floodplain (DF) versus those from the Mecsek Mts and Villányi Mts (MV).
4. Diploids (DP) versus polyploids (PP).

These pairs, although selected considering previous experience, are somewhat arbitrary and serve merely as a reference basis for the evaluation of characters. Thus, the higher the number of pairwise comparisons the more reliable the results on the taxonomic importance of characters will be. In addition to the rank order and significance tests, the method may provide information on the existence of the arbitrarily contrasted groups.

The output list starts with a table of variable statistics. This table gives preliminary information about the importance of characters. The means, maxima, minima and frequencies for each group are indicative of the usefulness of characters as early as the beginning of the analysis. The variable statistics for the pair HM-PL (Table 2) show, for example, that the width of the first leaf carries no significant information since the means are similar and there is not much difference between the minima and maxima. On the contrary, the length of the first leaf and that of the inflorescence, as well as their ratio are very different in the two groups. Highly significant χ^2 values are expected for these characters, especially if the differences between the corresponding extreme values are also high, as in case of character L.

This information is followed by the analysis of characters. The output and the assessment of results are illustrated by a detail of the output list produced by analyzing the pair HM-PL (Table 3). Of the possible threshold values the one most applicable is selected by the program. This is used in the further computations. The selection of the threshold is seen in case of multistate characters (12a, b, c). The discriminatory power of binary variables is calculated in a single step and is expressed by the magnitude of χ^2 .

Table 2
Variable statistics as printed by SCAGA
Scilla bifolia s.l. populations in 1983. First run. HM versus PL

| Char- acter | Mean | | Minimum | | Maximum | | Frequency | | | Threshold values | | | |
|----------------|--------|--------|---------|--------|---------|---------|-----------|-----|--------|------------------|-------|-------|-------|
| O | 94.881 | 82.300 | 50.000 | 48.000 | 180.000 | 125.000 | 160 | 100 | 100.00 | 150.00 | | | |
| W | 12.312 | 11.960 | 6.000 | 8.000 | 18.000 | 17.000 | 160 | 100 | 10.00 | 15.00 | | | |
| L | 76.956 | 69.250 | 10.000 | 30.000 | 161.000 | 95.000 | 160 | 100 | 6.00 | 8.00 | 10.00 | 12.00 | |
| Z | 96.312 | 90.710 | 50.000 | 55.000 | 160.000 | 130.000 | 160 | 100 | 100.00 | 150.00 | | | |
| A | 39.450 | 52.260 | 16.000 | 19.000 | 85.000 | 100.000 | 160 | 100 | 20.00 | 40.00 | 60.00 | | |
| I | 26.606 | 17.770 | 10.000 | 10.000 | 70.000 | 32.000 | 160 | 100 | 1.00 | 2.00 | 3.00 | | |
| N | 2.150 | 2.080 | 2.000 | 2.000 | 3.000 | 3.000 | 160 | 100 | 2.00 | | | | |
| P | 4.875 | 6.990 | 2.000 | 2.000 | 13.000 | 15.000 | 160 | 100 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 |
| H | 7.506 | 7.330 | 5.000 | 5.000 | 12.000 | 9.000 | 160 | 100 | 6.00 | 8.00 | | | |
| K | 5.750 | 6.000 | 4.000 | 6.000 | 6.000 | 6.000 | 160 | 100 | 6.00 | | | | |
| F | 13.056 | 11.920 | 8.000 | 8.000 | 20.000 | 15.000 | 160 | 100 | 1.00 | 2.00 | | | |
| S | 15.125 | 18.000 | 6.000 | 13.000 | 21.000 | 21.000 | 160 | 100 | 10.00 | 15.00 | 20.00 | | |
| M | 18.562 | 18.850 | 10.000 | 15.000 | 25.000 | 25.000 | 160 | 100 | 10.00 | 15.00 | | | |
| E | 1.250 | 2.200 | 1.000 | 1.000 | 3.000 | 3.000 | 160 | 100 | 1.00 | | | | |
| C | 31.500 | 21.600 | 18.000 | 18.000 | 54.000 | 36.000 | 160 | 100 | 18.00 | 36.00 | | | |
| Y | 1.125 | 1.000 | 1.000 | 1.000 | 2.000 | 1.000 | 160 | 100 | 1.00 | | | | |
| G | 1.875 | 1.200 | 1.000 | 1.000 | 2.000 | 2.000 | 160 | 100 | 1.00 | | | | |
| X | 1.375 | 1.400 | 1.000 | 1.000 | 2.000 | 2.000 | 160 | 100 | 1.00 | | | | |
| R | 1.250 | 1.600 | 1.000 | 1.000 | 2.000 | 2.000 | 160 | 100 | 1.00 | | | | |
| B | 1.750 | 1.200 | 1.000 | 1.000 | 2.000 | 2.000 | 160 | 100 | 1.00 | | | | |
| T | 1.750 | 1.200 | 1.000 | 1.000 | 2.000 | 2.000 | 160 | 100 | 1.00 | | | | |
| U | 1.625 | 1.400 | 1.000 | 1.000 | 2.000 | 2.000 | 160 | 100 | 1.00 | | | | |
| D | 1.750 | 1.200 | 1.000 | 1.000 | 2.000 | 2.000 | 160 | 100 | 1.00 | | | | |

Table 3

Detail of SCAGA output
Scilla bifolia s.l. populations in 1983. I. HM versus PL

| Character | Threshold | Contingency table | | | Proportions | | Tendency | chi ² | Significance of chi ² |
|-----------|-----------|-------------------|-----|-------|-------------|--------|----------|------------------|----------------------------------|
| | | A | B | A + B | | | | | |
| 12a. S | ≤10.00 | 20 | 0 | 20 | 0.1250 | 0.0000 | 1.0000 | 13.5417 | *** |
| | | 140 | 100 | 240 | 0.8750 | 1.0000 | | | |
| | | 160 | 100 | 260 | | | | | |
| 12b. S | ≤15.00 | 60 | 20 | 80 | 0.3750 | 0.2000 | 0.8000 | 8.8472 | ** |
| | | 100 | 80 | 180 | 0.6250 | 0.8000 | | | |
| | | 160 | 100 | 260 | | | | | |
| 12c. S | ≤20.00 | 140 | 80 | 220 | 0.8750 | 0.8000 | 0.8750 | 2.6591 | |
| | | 20 | 20 | 40 | 0.1250 | 0.2000 | | | |
| | | 160 | 100 | 260 | | | | | |
| 13a. M | ≤10.00 | 9 | 0 | 9 | 0.0562 | 0.0000 | 1.0000 | 5.8267 | * |
| | | 151 | 100 | 251 | 0.9437 | 1.0000 | | | |
| | | 160 | 100 | 260 | | | | | |
| 13b. M | ≤15.00 | 47 | 35 | 82 | 0.2937 | 0.3500 | 0.7062 | 0.9018 | |
| | | 113 | 65 | 178 | 0.7062 | 0.6500 | | | |
| | | 160 | 100 | 260 | | | | | |
| 14. E | ≤ 1.00 | 140 | 40 | 180 | 0.8750 | 0.4000 | 0.8750 | 65.1805 | *** |
| | | 20 | 60 | 80 | 0.1250 | 0.6000 | | | |
| | | 160 | 100 | 260 | | | | | |
| 15a. C | ≤18.00 | 60 | 80 | 140 | 0.3750 | 0.8000 | 0.8000 | 44.7262 | *** |
| | | 100 | 20 | 120 | 0.6250 | 0.2000 | | | |
| | | 160 | 100 | 260 | | | | | |
| 15b. C | ≤36.00 | 140 | 100 | 240 | 0.8750 | 1.0000 | | | |
| | | 20 | 0 | 20 | 0.1250 | 0.0000 | | | |

The groups specific by the user are denoted by A and B. All individuals are assigned to either group based on the threshold for each variable. After the threshold a contingency table and a relative frequency table follow. For character 12a (Table 3) the interpretation of these tables is as follows:

| Thresh- old | Contingency table | | | Relative frequency table | |
|----------------|-------------------|-----|-----|-----------------------------|-----|
| ≤10 | 20 | 0 | 20 | 0.125 | 0.0 |
| >10 | 140 | 100 | 240 | 0.875 | 1.0 |
| Total | 160 | 100 | 260 | | |

As seen, the number of seeds in all individuals of group B exceeds the threshold. The relative frequency table indicate the proportion of character states in the groups. The highest proportion occurs in cell 4, as all individuals in group B fall into this category. Such a maximum proportion is termed 'tendency' which means that a given state of a character in a group is maximum over the four cells. In other words, the corresponding locality tends to 'prefer' that character state.

In the output line the value of χ^2 follows. It is the expression showing the magnitude of the discriminatory power of the character for the given threshold. Then, the significance level is indicated:

* significant ($P = 0.05$)

** highly significant ($P = 0.01$)

*** very highly significant ($P = 0.001$)

The relationship between χ^2 and tendency is best illustrated with characters 12a and 14 in Table 3. Although the χ^2 values are very highly significant in both cases, a lower tendency is associated with a higher χ^2 (0.875 with 65.1) in case of character 14 whereas a high tendency (1.0) is associated with a lower χ^2 (13.5) for character 12a. Thus, no generally valid relationship may be established between χ^2 and tendency.

The tendency of 12a is higher than that of 14, as its distribution according to the selected threshold is unbalanced in both A and B (in B the proportion of frequencies being 0 : 1.0). As a result, the highest tendency (1.0) will be associated with the lower right cell of the table. In the other group the distribution is also unbalanced, seed number exceeding the threshold predominates and the resulting χ^2 is low. In case of character 14 the distribution within A is similar to that for character 12a, but in B the distribution is more even. Therefore, the tendency is lower than in 12a. Here, in the two groups most individuals fall into the opposite sides of the threshold (upper left and lower right cells). This is the reason for the high discriminatory power expressed by the relatively high χ^2 value.

Table 4 exemplifies the statistical summary overlooking each step of the analysis.

In the upper part of the table the next character is identified which is selected based on the highest χ^2 (or tendency, if either group is empty). The actual threshold and the χ^2 value (if not zero) are also given.

Then, three complementary contingency tables are printed. These contain the frequency distribution of individuals for the character actually chosen. These frequencies refer to the "mountain origin versus flatland origin" as contrasted with "below threshold versus over threshold" for the individuals assigned up to this step to either group A* (mountain origin estimated), group B* (qualified as being of flatland origin) or the waiting list.

The lowest part shows how the problem of recognizing groups is solved according to all characters selected so far. Under the heads "hit probability" and "error probability" percentages of actual groups (A and B) assigned to A* and B* in the previous steps are listed. For the sake of completeness the threshold for the last character is shown again. Then, the weighted averages of hit and error probabilities for the two groups follow. In this study this is an arithmetic mean because any localities to be compared are given similar weight. The percentages of individuals still on the waiting list after the completion of this step are also printed.

Table 5 was constructed so as to give information about the role of the actually selected character and the goodness of assignments performed. The values printed in the upper and lower parts of the table are highly informative, especially if we follow all output lists similar to the one shown in Table 5.

The analysis stops when the hit probability is close enough to 100%, or if the groups become empty. In most cases, depending on the number of characters, the stopping condition is achieved in three to ten steps. The output is continued with a summary of steps characterized with the threshold of most typical variables, the number of individuals assigned to either group and the hit probabilities for each step. The total number of individuals categorized and the averages of hit probabilities are listed last (Table 5).

Based on different pairwise comparisons it is possible to make a rank order of characters which may prove useful in taxonomic studies.

Table 4*Printout from one step of SCAGA analysis**Scilla bifolia* s.l. populations in 1983. I. Group. HM versus PL. Step 2.Max χ^2 value obtained for character 13: Seed numberCriterion of assignment: >20 ; χ^2 : 37.5

Cases assigned in this step to Group A*

The distribution of individuals in categories up to step 2 based on character 13

| | A* category | | | B* category | | | Waiting | | |
|-----------------|-------------|----|-----|-------------|---|---|---------|----|----|
| | A | B | S | A | B | S | A | B | S |
| Below threshold | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Over threshold | 160 | 40 | 200 | 0 | 0 | 0 | 0 | 60 | 60 |
| Total | 160 | 40 | 200 | 0 | 0 | 0 | 0 | 60 | 60 |

| Hit and error probabilities | | | | Discriminance threshold | Average hit and error probabilities | Proportion of waiting individuals | | |
|-----------------------------|-----|------|-----|-------------------------|-------------------------------------|-----------------------------------|-----|------|
| A | | B | | | | A | B | |
| A* | B* | A* | B* | | | | | |
| 100.0 | 0.0 | 40.0 | 0.0 | 20 | 50.0 | 20.0 | 0.0 | 60.0 |

Table 5*Summary of a SCAGA analysis**Scilla bifolia* s.l. populations in 1983. I. Group. HM versus PL

| Step | Character | Threshold | Group | Nr. of assigned individuals | Nr. of reassigned individuals | Hit % |
|------|-----------|---------------|-------|-----------------------------|-------------------------------|-------|
| 1. | G | > 1.00 | A* | 160 | | 43.7 |
| 2. | S | > 20.00 | A* | 40 | | 50.0 |
| 3. | O | ≤ 150.00 | B* | 60 | 40 | 80.0 |
| 4. | L | > 12.00 | B* | 40 | | 100.0 |

| Classification after four steps | | | | | | Average hit % |
|---------------------------------|-----|-----|-----|-------|-------|---------------|
| | A | B | S* | A | B | |
| A* | 160 | 0 | 160 | 100.0 | 0.0 | |
| B* | 0 | 100 | 100 | 0.0 | 100.0 | |
| S | 160 | 100 | 260 | | | 100.00 |

Results

The summary of significant characters found in four runs of SCAGA is Table 6. Within the category of very highly significant characters further subdivisions are made based on the χ^2 values:

$$\chi^2 > 100 = \bigcirc, \quad \chi^2 > 50 = \otimes, \quad \chi^2 > 30 = \bullet$$

First, the results of the four pairwise analyses are evaluated, then the comparative assessment follows. As most of the correct assignments are made in step 1 in every case, the characters first selected reveal the most important difference between the given groups. Therefore,

Table 6
The significance of characters in the four runs of SCAGA

| Char- acter | HM vs. PL | | | TD vs. NM | | | DF vs. MV | | | DP vs. PP | | | Occur- rence |
|----------------|----------------|--------------|--------------|--------------|---|---|-----------|---|---|-----------|---|---|-----------------|
| 1. O | ≤100 *** PL | ≤150 ** NM | ≤100 ** MV | ≤100 ○ DP | 4 | | | | | | | | |
| 2. W | ≤150 * PL | | | | | | | | | | | | |
| 3. L | — | — | — | — | — | — | — | — | — | — | — | — | |
| 4. Z | ≤100 *** PL | >100 *** NM | ≤100 *** MV | — | 3 | | | | | | | | |
| 5. A | ≤20 ** PL | | >40 * DF | >20 *** DP | 3 | | | | | | | | |
| | >40 ○ PL | — | ≤60 *** MV | >40 ○ DP | | | | | | | | | |
| | ≤60 *** HM | | | | | | | | | | | | |
| 6. I | — | — | — | — | | | | | | | | | |
| 7. N | — | 2 *** TD | 2 * MV | — | | | | | | | | | |
| 8. P | >4 *** PL | ≤4 *** TD | >4 ** DF | >4 ⊗ DP | 4 | | | | | | | | |
| | ≤6 *** HM | ≤6 *** TD | ≤6 ** MV | ≤6 ○ PP | 4 | | | | | | | | |
| | ≤8 *** HM | ≤8 *** NM | ≤8 * DF | ≤8 *** DP | 4 | | | | | | | | |
| | | ≤8 *** TD | ≤8 * MV | | | | | | | | | | |
| 9. H | — | ≤6 *** NM | ≤6 * DF | ≤8 *** DP | 3 | | | | | | | | |
| | | ≤8 *** TD | ≤8 * MV | | | | | | | | | | |
| 10. K | — | — | — | — | | | | | | | | | |
| 11. F | — | — | — | — | | | | | | | | | |
| 12. S | >10 *** PL | >10 *** TD | — | 10 *** PP | 3 | | | | | | | | |
| | >15 ** L | ≤15 *** TD | — | 15 ● DP | | | | | | | | | |
| | | | | 20 ● PP | | | | | | | | | |
| 13. M | >1.0 * PL | >1.5 * NM | >1.0 *** DF | >1.5 ** DP | 4 | | | | | | | | |
| 14. C | 18 ○ PL | 36 *** TD | — | 18 ● DP | 3 | | | | | | | | |
| | | 36 *** NM | | 36 *** PP | | | | | | | | | |
| 15. E | grained *** HM | — | — | grained ● PP | | | | | | | | | |
| 16. Y | early *** PL | early *** NM | — | early *** DP | 3 | | | | | | | | |
| 17. G | green ● HM | — | redd. ○ DF | green ● PP | 3 | | | | | | | | |
| 18. X | — | early *** NM | late *** MV | early ○ PP | 3 | | | | | | | | |
| 19. R | thin *** HM | — | thick *** MV | thin ● PP | 3 | | | | | | | | |
| 20. B | green ⊗ PL | — | — | blue ● PP | | | | | | | | | |
| 21. T | white ⊗ PL | — | — | blue ● PP | | | | | | | | | |
| 22. U | redd. *** HM | redd. *** TD | — | redd. ● PP | 3 | | | | | | | | |
| 23. D | light ⊗ PL | — | — | dark ● PP | | | | | | | | | |

Signif.

ratio HM : PL = 6 : 11 TD : NM = 6 : 7 DF : MV = 5 : 8 DP : PP = 7 : 13

the rank order of significant characters according to χ^2 is a very important source of information in the first step. We are informed about the number of characters required for discriminating between the groups.

1. Group HM versus group PL

The discriminative characters in the first four steps, the number of individuals categorized in each step and the hit probabilities are as follows:

| | | | |
|--------|-----------|----------|-----------------------|
| Step 1 | G (green) | HM — 160 | 43.7% |
| Step 2 | S (>20) | HM — 40 | 50.0% |
| Step 3 | O (≤150) | PL — 60 | 80.0% (40 reassigned) |
| Step 4 | L (>12) | PL — 40 | 100.0% |

Based on the first step the rank orders of characters for the separate groups are:

| Group HM | χ^2 | Group PL | χ^2 |
|----------------|----------|-----------------|----------|
| G (green) | 118.4 | B (green) | 74.9 |
| E (grained) | 65.1 | T (white) | 74.9 |
| R (thin) | 31.8 | D (light ochre) | 74.9 |
| P (≤ 6) | 26.9 | C (= 18) | 44.7 |
| | | A (>40) | 30.8 |
| | | P (>6) | 28.6 |

In the two groups the χ^2 values are equally high, except character G, but the characters themselves are different. The individuals living in the mountainous areas are characterized by green stem, variable shaped and grained elaiosomes, thin root and six or less flowers. In the other group the buds are grayish-green, the tepals have white base, the inflorescence is longer than 40 mm, chromosome number is always 18 and seed colour is light ochre. Although the stems in group HM may have brown spots, the whole stem is not reddish. The occurrence of green stems can not be explained on the basis of shady habitat conditions since some flat-land populations also live in shady forests along the rivers. The number of flowers with a threshold of 6 may well be used for discrimination. It is of course only statistically valid. Moreover, this character is much influenced by age. Our experience suggests that a characteristic flower number will be manifested only under normal circumstances from 5–8 years old bulbs.

As the analysis proceeds, characters applicable to discriminate based on small differences will be emphasized. In our case, after the first 'rough' classification, which usually requires qualitative characters, the quantitative variables will become important. These are: leaf length and the ratio of leaf length and width. Table 6 also shows the most typical threshold values for the quantitative characters.

2. Group TD versus group NM

The discriminative characters, the number of individuals categorized using these characters and the hit probabilities are:

| | | | |
|--------|------------------|----------|-----------------------|
| Step 1 | M (>1.0) | TD — 100 | 50.0% |
| Step 2 | O (≤ 150) | NM — 20 | 66.7% (40 reassigned) |
| Step 3 | Z (≤ 150) | NM — 40 | 100.0% |

The highly ranked characters in Step 1 are:

| Group TD | χ^2 | Group NM | χ^2 |
|----------------|----------|-----------|----------|
| S (>10) | 24.0 | X (early) | 24.0 |
| U | 24.0 | C (= 36) | 24.0 |
| C (= 36) | 24.0 | Z (>100) | 16.6 |
| N (= 2) | 20.8 | | |
| P (≤ 6) | 20.8 | | |
| H (≤ 8) | 13.2 | | |

The goal of this comparison was to see whether the populations from the different mountainous regions of the country differ from each other and if so, what characters are

responsible for this difference. This problem is important, because there are some views suggesting that the populations in these two regions are taxonomically different (SPETA 1972). The proportion of significant characters in the two groups is almost identical. There is no extremely significant character, i.e. the χ^2 values are of similar magnitude. Only the distribution of characters in the two groups is different. The TD populations are characterized by red fruit with more than 10 seeds in each, 6 or less flowers and small tepals. Of the NM populations early withering and long stem are characteristic. The number of chromosomes is significant in both groups, so the mountain populations are, in general, polyploid.

3. Group DF versus group MV

The discriminative characters, the number of categorized individuals and the hit probabilities are as follows:

| | | | |
|--------|------------------|---------|--------|
| Step 1 | G (reddish) | DF — 80 | 50.0% |
| Step 2 | O (≤ 150) | MV — 20 | 75.0% |
| Step 3 | W (≤ 15) | MV — 20 | 100.0% |

The significant characters in the first step are:

| Group DF | χ^2 | Group MV | χ^2 |
|-----------------|----------|--------------------|----------|
| G (reddish) | 37.5 | R (thick) | 16.6 |
| M (> 1.0 mm) | 24.8 | X (late) | 16.6 |
| P (> 4) | 10.6 | A (≤ 60 mm) | 11.7 |
| | | Z (≤ 100 mm) | 10.8 |

Early observations allowed for the conclusion that the *Scilla* populations at higher altitudes in the Mecsek Mts and Villányi Mts are taxonomically different from those living in the valleys. They have less flowers, smaller size and thicker roots, although the chromosome number and seed colour are similar in both cases. It is likely that the mountain populations are remnants of an old population distributed along the Danube river. After the elevation of the mountains they became adapted to the new environmental conditions.

The above figures show that the χ^2 values are considerably different in the two groups. Group DF is characterized by red stems and flowers more than 4, but these characters are not discriminative for the other group in which the seeds are smaller. The smaller size in group MV is demonstrated by the strong significance of stem length < 100 mm.

4. Group DP versus group PP

The discriminative characters, the number of individuals categorized and the hit probabilities are:

| | | | |
|--------|-------------|----------|------|
| Step 1 | C (= 18) | DP — 140 | 50% |
| Step 2 | I (> 3) | PP — 120 | 100% |

It is seen at a glance that ploidy level is of fundamental importance so that two steps were sufficient to classify the individuals.

The decisive characters in Step 1 are:

| Group DP | χ^2 | Group PP | χ^2 |
|------------------|----------|-----------------|----------|
| C (= 18) | 260.0 | G (green) | 139.2 |
| P (>4) | 71.3 | R (thin) | 139.2 |
| S (>15) | 38.7 | B (blue) | 191.0 |
| A (>40) | 32.6 | T (blue) | 191.0 |
| H (≤ 8) | 20.9 | U (reddish) | 191.0 |
| O (≤ 100) | 13.7 | D (dark brown) | 191.0 |
| | | E (grained) | 99.0 |
| | | P (≤ 6) | 55.6 |
| | | S (≤ 15) | 44.5 |
| | | C (= 36) | 25.0 |

The magnitude of χ^2 and the differences between the groups are greatest among the four pairs. It is surprising that group DP is characterized by relatively few but exclusively quantitative characters, whereas in group PP the qualitative characters are of primary importance. It seems that group PP represents a more clearly defined group: the average χ^2 is high and the number of significant characters is also large. Group DP, which is composed mainly of lowland populations, is characterized by chromosome number $2n = 18$, whereas the chromosome number ($2n = 36$) in group PP is significant only at a low level because of the presence of the hexaploid ($2n = 54$) population.

The results of our pilot studies are confirmed by the importance of character I in step 2. The ratio of stem length to the axis of inflorescence proved to be a typical value for all mountain populations that are usually polyploid. These results are in good agreement with the results of the first three runs: group DP is characterized by many flowers and seeds, smaller tepals and longer inflorescence axis. The predominantly mountain populations in group PP are best described by the quantitative characters listed above.

Discussion

Exceedingly high χ^2 values were obtained almost always for the quantitative characters. It means that the quantitative characters are more useful for taxonomic purposes, especially for distinguishing between groups DP and PP. Characters W, L, K and F are less important in the first steps, but later they may still prove significant. The expected strong significance of characters O, L, Z and A was revealed mainly in steps 2, 3 or 4. Characters K and F were never found to be significant, they may be omitted from future studies. Characters significant in all pairwise comparisons were P, O and M. The others are decisive only in two or three cases. The rank order of characters (Table 7) on the basis of χ^2 values does not reflect by itself a real order, because most characters are discriminative for only one or two pairs. Nevertheless, some conclusions may be drawn from this table.

The explanation for the high χ^2 values is partly the a priori selection of pairs, e.g., the high value for $2n = 18$ is a result of the classification based on

ploidy level. Thus, the primary importance of chromosome number is proved. The blue colour of tepals is characteristic of the polyploids. These, mainly mountain populations are characterized by medium or light blue tepals. This colour may be seen even on the androeceum and gynoecium. These tepals are usually longer (8–12 mm) and wider (3 mm) than those of the lowland indi-

Table 7
The rank order of characters based on χ^2

| No. | Character and character state | chi ² | Significant for group |
|-----|-------------------------------|------------------|-----------------------|
| 14. | Chromosome no.: $2n = 18$ | 260.0 | DP |
| 20. | Bud colour; blue | 191.0 | PD |
| 17. | Stem colour; green | 139.2 | PP |
| 19. | Root; thin | 139.2 | PD |
| 17. | Stem colour; green | 118.4 | HM |
| 15. | Elaiosome; grained | 99.0 | PP |
| 20. | Bud colour; green | 74.9 | PL |
| 21. | Tepal colour; white on base | 74.9 | PL |
| 23. | Seed colour; light ochre | 74.9 | PL |
| 8. | Flower number; 4 | 71.3 | DP |
| 8. | Flower number; 6 | 55.6 | PP |
| 12. | Seed number; 20 | 44.5 | PP |
| 12. | Seed number; 15 | 38.7 | DP |
| 17. | Stem colour; reddish | 37.5 | DP |

viduals of which the tepals are dark violet blue with a sharply distinct white basal spot, shorter (5–8 mm) and narrower (1.5 mm). The septa of ovary occur as white strips.

Root thickness is also useful in distinguishing between groups DP and PP. All individuals collected along the Danube are characterized by relatively few, strong, heavy, chalk-white roots. In case of the other lowland populations the roots are thin and whitish, a bit transparent. The roots of mountain populations, especially those from Nagyhideghegy, are very thin, dense and transparent. The red colour of stalk is less discriminative for the lowland populations, whereas the green colour is more discriminative for the mountain populations. Characters C, B, T, D, P, S and G are discriminative for group DP. The polyploids are characterized by B (blue), R, E, P (6) and S (<20). Three characters; P, S and G are strongly discriminative in two cases. It is striking that among the $\chi^2 \geq 30$ values neither length nor width data are represented, the variability of these characters decreases their discriminatory power.

The comparison of diploids and polyploids (Table 8) revealed the characters important in group assignments. The identification numbers of those

variables significant for at least three groups are underlined. It was established that character P is important in case of the mountain populations, the threshold being 6. Less flowers are found in the polyploids, more in the diploids. Character S is discriminative for the mountain populations (<15), and for the lowland ones, too (>15). Characters R and U are also typical. The lowland

Table 8

The comparison of mountain and flatland populations

| HM | NM | TD | MV |
|----------------|-----------------|---------------|---------------------|
| A ≤ 60 | +Z >100 | N 2 | +Z 100 mm |
| P $\leq 6-8$ | +N >2 | P $<6-8$ | A 60 mm |
| E grain | +P >6 | H ≤ 8 mm | P ≤ 6 |
| G green | +H >6 | S 10-15 | +N 2 |
| R thin | +M >15 mm | U reddish | +R thick |
| U reddish | +Y early | | +X late |
| B blue | +X early | | |
| S 10-15 | | | |
| PP | PL | DF | DP |
| P $\leq 6-8$ | O ≤ 100 mm | A >40 | O $\leq 100-150$ mm |
| S $\leq 10-20$ | Z ≤ 100 mm | P $>4-6$ | A >40 mm |
| C ≤ 36 | A >40 mm | H >6 | P $\leq 4-8$ |
| E grain | P >4 | M >10 mm | H ≤ 8 mm |
| G grain | S $>10-15$ | G reddish | S >15 |
| X early | M >10 mm | | M ≤ 15 mm |
| R thin | C 18 | | C 18 |
| B blue | Y early | | Y early |
| T blue | B green | | |
| | T white | | |
| | D spotted | | |
| | D ochre | | |

+ Extremely high χ^2 -values

populations, on the other hand, are unambiguously differentiated by characters A (>40 mm), P (>4) and M (1.0 mm on the average). There are also some differences between the mountain populations which may be considered as separate taxa. The populations from Mátra and Bükk Mts. have more than two leaves, large seeds (>1.5 mm) and tepals (>6 mm), and early date of flowering and withering. The Mecsek Mts. population is characterized by 100 mm long stalks, constantly two leaves, late leaf withering and thick roots. These findings serve as a basis for future taxonomic studies.

For the classification of the *Scilla* populations in Hungary, the rank order of strongly discriminative qualitative characters is given here. The order is:

stem colour
 bud colour
 root thickness
 elaiosome shape
 tepal colour
 seed colour
 date of flowering
 date of complete leaf withering

The quantitative characters may be ranked as:

number of flowers
 number of seeds
 length of inflorescence axis
 length of the first leaf
 seed length

The numbers of individuals assigned by SCAGA into groups provide some useful information on the taxonomy of the aggregate:

| | | | |
|-------------|------------|------------|-------------|
| 1. HM = 160 | 2. TD = 60 | 3. DF = 60 | 4. DP = 140 |
| PP = 100 | NM = 60 | MV = 40 | PP = 120 |
| Total: 260 | 120 | 100 | 260 |

Based on the above mentioned characters it was possible to divide the sample into two groups of similar size in each comparison:

62–38% 50–50% 60–40% 54–46%

The present study confirmed the results of preliminary studies. The separation between mountain and lowland populations and the difference according to ploidy level are clearly shown. Furthermore, it seems possible to subdivide even the mountain populations. The strong separation of populations in the Hungarian Central Range suggests that they represent distinct taxa. Run 3, the comparison of TD and NM populations, is especially interesting in this regard. The results of run 3 emphasize the importance of future studies of these populations.

The present results are in good agreement with those obtained by multivariate analysis (KERESZTY and PODANI 1985). Almost the same characters proved to be discriminative and the grouping according to the geographical localities was also clear in the study.

Runs 1 and 4 support our previous view that the populations along the Hungarian section of Danube may be identified as *Scilla vindobonensis* Speta (SPETA 1977).

The results also suggest that the populations of different geographical regions of Hungary represent a local taxon each, especially those in the Bükk, Velencei and Mecsek Mountains. The possibility of further subdivisions in certain groups may not be excluded. In addition to the outcomes of SCAGA analysis, the present study determined the direction of our future work concerning the taxonomy of the *Scilla bifolia* aggregate. The applicability of our method is supported by the fact that the separate analysis of data from two subsequent years yielded similar results, therefore, in this paper only those based on the 1983 data are presented.

It is the authors' purpose to continue this study by incorporating other methods of biostatistics. SCAGA served as a preliminary to the more detailed analysis of characters.

Botanical conclusions

The SCAGA analysis of 23 characters describing 260 individuals of the *Scilla bifolia* Aggregate collected from 17 localities in Hungary may be summarized as follows:

1. Five quantitative and eight qualitative characters turned out to have high discriminatory power if all localities are considered.
2. The qualitative characters are primarily important in case of diploids.
3. In all four pairwise comparisons of groups the length of the first leaf, the number of flowers and the length of dry seeds proved to be significant.
4. Some characters are apparently useless for taxonomic purposes, others are significant only in certain comparisons.
5. The threshold for flower and seed number and inflorescence axis characteristic of each group were determined.
6. It was proved by the χ^2 values that the quantitative characters are of secondary (or local) importance in the pairwise comparisons.
7. The rank order of characters for the groups of diploids and polyploids was clearly determined by the analysis.

The present results support and agree with former statements on the taxonomy of the *Scilla bifolia* Aggregate in Hungary.

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The authors are grateful to Dr. J. PODANI for his helpful suggestions, and to Mrs. M. LOMJÁNSZKI for her technical assistance.

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ADICIONES AL CATÁLOGO DE LAS PALMAS DE CUBA

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The taxonomic position of the genus *Hemithrinax* is reconsidered by the authors. Its separation from *Thrinax* based upon an only differential morphological feature seems to be insufficient. For this reason the authors suggest to consider it as a subgenus of *Thrinax*. This new state must be followed by a number of new combinations.

Two further new palms of the genus *Coccothrinax* Sarg. has been found in the south-central and south-eastern regions of Cuba. *Coccothrinax fagildei* Borhidi et Muñiz sp. n. is living in the dry coastal limestone dog-tooth areas of the terraces of Verraco, East of Santiago de Cuba. It is confined taxonomically to *C. elegans* Muñiz et Borhidi from the Sierra Maestra. The other new species, *Coccothrinax trinitensis* Borhidi et Muñiz sp. n. is an endemic of the montane limestone karsts (mogotes) of the Pico Potrerillo and surroundings (Sierra de Escambray) above the city Trinidad. It is related to *C. acunana* León from the montane belt of the Sierra Maestra.

Introducción

En el año 1983 fue publicado un catálogo fitotaxonómico crítico de las palmas de Cuba, incluidas sus claves analíticas (MUÑIZ y BORHIDI 1983).

Desde entonces algunas adiciones y modificaciones ha surgido la necesidad de hacer de este catálogo.

La posición taxonómica del género *Hemithrinax* fue reconsiderado por los autores, porque su separación del género *Thrinax* estaba basado en un solo caracter diferencial morfológico, lo que parecía insuficiente para mantenerlo como género distinto. Por esta razón sugerimos clasificar el *Hemithrinax* como subgénero de *Thrinax*. Esta consideración hace necesario de crear un número de combinaciones nuevas. La taxonomía del género *Thrinax* en Cuba se ha modificado en la forma siguiente:

THRINAX Sw. Prodr. 4: 57. 1788

- 1 a Espádices paniculado-ramificados, flores bracteádas, anteras con filamentos subulados, exertos (subgénero *Thrinax*) 2
- b Espádices 2-ramificados, flores sin brácteas, anteras sentadas o subsentadas (subgénero *Hemithrinax*) 4
- 2 a Vaína profundamente excisa en forma de V en el ápice; hojas verdes en ambas caras; flores y frutos con un pedicelo de 1-2 mm de largo 1. **T. radiata**
- b Vaína lingüiforme en el ápice; hojas blancuzcas en el envés, mayormente con rayas de puntos blancos; flores y frutos subsentados a sésiles 2. **T. Morrisii**
- 3 a Fruto de 4-9 mm de diámetro; inflorescencias de 50-80 cm de largo (sección *Hemithrinax*) 4

- b Fruto de 13–15 mm de diámetro; inflorescencias de 150–200 cm de largo (sección *Macrocarpae*) 3. **T. rivularis**
 aa Palma de 6–8 m de alto, pecíolo mas largo que la vaina var. **rivularis**
 bb Palma de 1–4 m de alto, pecíolo de igual largo o menos que la vaina . var. **savannarum**
 4 a Vaina con una capa gruesa de lana; limbo de la hoja orbicular de 120–140 cm de diámetro, con 60–70 segmentos 4. **T. compacta**
 b Vaina glabra con fibras gruesas; limbo de la hoja semiorbicular de 50 cm de diámetro con 30–36 segmentos 5. **T. Ekmaniana**

Subgenus: THRINAX

Sectio *Thrinax*

- 1.1. **Thrinax radiata** Lodd. ex Schult. in Linn. Syst. Veg. sec. 7 (2): 1301. 1830.
 (Syn.: *T. Wendlandiana* Becc. Webbia **2**: 285. 1907; *T. parviflora* auct. e.g. Alain in Flora de Cuba Suppl. 29. 1969. non Sw.; *T. Martii* Griseb. et Wendl. p.p. ex Griseb. Cat. Pl. Cub. 1866: 221.; *T. floridana* Sarg. Bot. Gaz. **27**: 84. 1899.)
 1.2. **Thrinax Morrisii** Wendl. in Gard. Chron. (ser. 3.) **11**: 104. fig. 20. 21. 1892.
 (Syn.: *T. microcarpa* Sarg. in Gard. For. **9**: 162. 1896.; *T. keyensis* Sarg. Bot. Gaz. **27**: 86. 1899.; *T. bahamensis* O. F. Cook in Northrop Mem. Torr. Bot. Cl. **12**: 20. 1902.; *T. Drudei* Becc. Webbia **2**: 269. 1907.; *T. punctulata* Becc. l.c. 280. *T. Ekmanii* Burret Kungl. Sv. Vet-Akad. Handl. ser. 3, **6** (7): 27. 1929.)

Subgenus: HEMITHRINAX (Hook. f.) Drude 1889 Nat. Pflanzenfam. II/3:34. (Basionymon: *Hemithrinax* pro gen. Hook. f. in Benth. et Hook. Gen Plant. **3**: 930. 1883)

Sectio: **Macrocarpae** (León ex Muñiz) Borhidi et Muñiz **comb. nova**
 (Basionymon: *Macrocarpae* León ex Muñiz in Muñiz et Borhidi, Acta Bot. Acad. Sci. Hung. **28**: 312. 1982. Syn.: *Macrocarpae* León in Mem. Soc. Cub. Hist. Nat. **15**: 383. 1941. nom. illeg.)

- 1.3. **Thrinax rivularis** (León) Borhidi et Muñiz **comb. nova**
 (Basionymon: *Hemithrinax rivularis* León Mem. Soc. Cub. Hist. Nat. **15**: 380. 1941.)
 1.3.1. var. **rivularis**
 1.3.2. var. **savannarum** (León) Borhidi et Muñiz **comb. nova**
 (Basionymon: *Hemithrinax savannarum* León Mem. Soc. Cub. Hist. Nat. **15**: 381. 1941.; Syn.: *Hemithrinax rivularis* var. *savannarum* Muñiz in Muñiz et Borhidi Acta Bot. Acad. Sci. Hung. **28**: 312. 1982.)

Sectio: *Hemithrinax*

(*Microcarpae* León l.c. 383. 1941.)

- 1.4. **Thrinax compacta** (Griseb. et Wendl.) Borhidi et Muñiz **comb. nova**
 (Basionymon: *Trithrinax compacta* Griseb. et Wendl. ex Griseb. Cat. Pl. Cub. 1866: 221.; Syn.: *Hemithrinax compacta* [Griseb. et Wendl.] Hook. f. in Benth. et Hook. Gen. Plant. **3**: 931. 1883.)

1.5. *Thrinax Ekmaniana* (Burret) Borhidi et Muñiz **comb. nova**

[Basionymon: *Hemithrinax Ekmaniana* Burret in Kungl. Svensk. Vet.-Akad. Handl. 6 (7): 9. 1929.]

Dos especies nuevas del género *Coccothrinax* Sarg. en Cuba

Como se ha supuesto por los autores de este artículo, la variabilidad taxonómica del género *Coccothrinax* no es cabalmente conocida. Este supuesto está confirmado por el descubrimiento reciente de dos especies nuevas de este género, que describimos adicionalmente en este artículo, como complemento del mencionado catálogo.

Con estas dos nuevas especies el número de especies del género *Coccothrinax* existentes en Cuba, aumenta a 36, de las cuales 35 son endémicas de Cuba.

***Coccothrinax fagildei* Borhidi et Muñiz sp. nova**

Palma gregaria, usque ad 5–6 m alta; caules e basi pluries abeuntes, tenues, cylindracci, superne paullo curvati, 4.5–6 cm crassi in diametro. Vagina frondis 25–30 cm longa, 5–7 cm lata, fibris 0.5–1 mm crassis, lanatis satis dense intertexta, pars libera oblongo-triangularis, 6–10 cm longa, apice obtusa, et fibroso-tomentulosa, sine apicibus liberis acutis. Petiolum cum vagina 35–45 cm longum, pars libera petioli 18–22 cm longa, sub apice 1 cm lata, supra convexa, subtus applanata, longitudinaliter nervosa et cera induta; apice ligulis binis, superiore ovatis 6–8 mm longis, inferiore anguste lanceolatis, acutis, 3–5 mm longis suffultum. Lamina frondis semiorbicularis vel 2/3-orbicularis basi obtusa vel breviter rotundata, 28–35 cm longa et 40–45 cm lata, segmentis 20–25 praedita, segmenta centralia 28–30 cm longa, 2–2.5 cm lata, basi usque ad 7–8 cm longe connata superne abrupte angustata et in apices 9–12 cm longos excurrentia; lateralia linearis-subulata, 18–20 cm longa et 3–5 mm lata, omnia supra plana et viridia, opaca, nervis primariis, secundariis atque tertiariis conspicuis, non prominulis suffulta, subtus indumento adpresso permanentemente argentea, nervosa et punctis prominulis, sub indumento pallide luteis, dense punctulata.

Inflorescentiae axillares, recurvatae, e racemis 3–4 partialibus compositae, 60–70 cm longae, pedunculo 40–50 cm longo; bracteae lanceolatae, 4–8 cm longae, ellipticae, apice tomentosae. Inflorescentiae partiales 10–12 cm longae, simplice ramificatae. Flores 2.5–4 mm longe pedicellati, bracteolae pedicellis aequilongae vel paullo longiores, lobi perianthii, 1–1.5 mm longi, basi breviter connati, triangulares, filamentis latiores. Stamina 9, filamenta linearia, lobis perianthii aequilonga, antherae sagittatae, versatiles. Ovarium oblongo-ovatum, levissime punctulatum; stylus ovario aequilongus vel longior, apicem versus valde — usque ad 1–1.2 mm — dilatatus. Fructus globosus, 8–10 mm in diametro, pericarpium papyraceum, punctis prominulis regulariter et dense dispositis rugulosum. Semen osseum, 6–7 mm in diametro, depresso-globosum, sulcis 5 usque ad basem directis sulcatum.

Holotypus: MUÑIZ 15021 HAC; Cuba, Prov. Santiago de Cuba; En diente de perro costero a 300 m del mar y 200 m al oeste del Rio Verraco. Col.: ONANEY MUÑIZ, J. A. FAGILDE CELEIRO, BRUNO GONZALEZ 25. 4. 1984.

Hanc speciem collectori sui, il.mo José Antonio FAGILDE CELEIRO, delectanti botanicae, dedicavimus.

Palma de hasta 5–6 m de alto con troncos agrupados saliendo desde las hondonadas de la piedra hueca, delgados y cilíndricos de 4.5–6 cm de diámetro, erguidos, ligeramente

encurvados hacia el ápice. Vaina de la hoja de 25–30 cm de largo y 5–7 cm de ancho, densamente entretejida de fibras finas de 0.5–1 mm de grueso, ligeramente lanudas, la parte libre oblongo-aovado u oblongo-triangular de 6–10 cm de largo con fibras finas tomentosas en el ápice, sin puntas libres. Pecíolo con la vaina de 35–45 cm de largo, la parte libre del pecíolo de 18–22 cm de largo, 1 cm de ancho debajo del ápice, convexo en el haz, aplanado, longitudinalmente venoso y ceroso en el envés. Lígulas 2 en el ápice del pecíolo, la superior semi-orbicular-aovada de 6–8 mm de largo, la inferior estrechamente lanceolada, aguda, de 3–5 mm de largo. Limbo de la hoja semi-orbicular o 2/3-orbicular, obtuso o brevemente redondeado en la base, de 28–34 cm de largo y 40–45 cm ancho; segmentos 20–25, los centrales de 28–30 cm de largo y 2–2.5 cm de ancho, abruptamente ensanchados hacia el ápice en una punta aguda de 9–12 cm de largo; los basales linear-subulados de 18–20 cm de largo y 3–5 mm de ancho, todos verdes mates y lisos en el haz con nervios primarios secundarios y terciarios visibles, no prominulos, con un indumento plateado denso y permanente, nervios y puntos densos y prominulos en el envés con puntos amarillos densamente dispuestos debajo del indumento.

Inflorescencia axilar, encurvada, compuesta de 3–4 racimos parciales, de 60–70 cm de largo con el pedunculo de 40–50 cm de largo; brácteas lanceoladas de 4–8 cm de largo, tomentosas en el ápice. Inflorescencias parciales de 10–12 cm de largo, simplemente ramificadas. Flores pediceladas con pedicelos de 2.5–4 mm de largo, bractéolas de igual largo, o poco más largos que el pedicelo. Lóbulos del periantio 6, de 1–1.5 mm de largo, brevemente connados en la base, triangulares, más anchos que los filamentos. Estambres 9, filamentos lineales, de igual largo que los lóbulos del periantio; anteras aflechadas, versátiles. Ovario oblongo-aovado, ligeramente punteado; estilo igual largo que el ovario, muy ensanchado hacia el ápice hasta 1–1.2 mm de ancho. Fruto globoso, de 8–10 mm de diámetro, el pericarpio papiráceo, con puntos prominulos dense dipuestos ruguloso. Semilla ósea, de 6–7 mm de diámetro, deprimido-globosa con surcos 5 profundos llegando hasta la base.

Holotipo: MUÑIZ 15021 HAC; Cuba, Prov. Santiago de Cuba. En diente de perro costero, a 300 m del mar y 200 m al oeste del Rio Verraco. Col.: ONANEY MUÑIZ, José Antonio FAGILDE CELEIRO y BRUNO GONZALEZ, 25. 4. 1984.

Esta especie—esta dedicada a su colector, José Antonio FAGILDE CELEIRO, presidente del Grupo de Aficionados a la Botánica de la prov. de Santiago de Cuba.

***Coccothrinax trinitensis* Borhidi et Muñiz sp. nova**

Palma humilis, usque ad 3–4 m alta; caulis cylindraceus, erectus, 6–10 cm crassus in diametro. Vagina frondis 32–38 cm longa, 11–15 cm lata, superne valde ampliata, fibris rigidis, 0.8–1.2 mm crassis dense intertexta, pars libera late ovata vel plerumque truncata, 6–7 cm longa, sine apicibus liberis acutis. Petiolum cum vagina 75–80 cm longum, pars libera petioli 40–45 cm longa, sub apice 1.5 cm lata, utrinque convexa. Ligula 1, triangulari-ovata, leviter 3-loba, 1.5–2 cm longa, apice acuta. Lamina frondis orbicularis vel 3/4-orbicularis, basi rotundata, 36–40 cm longa et 40–42 cm lata, segmentis 38–42 praedita, segmenta centralia 36–38 cm longa, 4.5–5 cm lata, basi usque ad 12–14 cm longe connata, superne leviter attenuata et in apices 8–9 cm longos excurrentia; lateralía lineari-subulata, 18–20 cm longa et 6–8 mm lata, omnia supra plana et viridia, opaca, nervis primariis, secundariis atque tertiariis conspicuis non prominulis, tertiariis saepe obsoletis suffulta, subtus pallide glaucescentia, sine indumento argenteo, punctis flavescentibus pallidis dense disposita, nervis primariis atque secundariis obscure prominulis, tertiariis nullis.

Inflorescentiae axillares, e racemis 2 partialibus, recurvatis compositae, 50–60 cm longae, pedunculo 20–30 cm longo, dense bracteoso; bractae pergamaceae, lineari-ovatae vel lineari-lanceolatae, 12–15 cm longae, apice leviter tomentosae, postremo glabrae. Inflorescentiae partiales 20–25 cm longae, valde ramosae, ramis lateralibus usque ad 30. Flores 1–2 mm longe pedicellati, bracteolae pedicellis breviores; lobi perianthii cca 1 mm longi, anguste triangulari-subulati, basi filamentis angustiores. Stamina 6–7, filamenta oblongo-triangularia, basi breviter connata, lobis perianthii longiora, antherae oblongo-ellipticae vel lineari-ellipticae non sagittatae. Ovarium late ovatum, levissime punctulatum, stylus ovario aequilongus, apicem versus leviter dilatatus. Fructus depresso globosus, 7–8 mm in diametro; pericarpium papyraceum, punctis prominulis dense dispositis rugulosum. Semen osseum, 4.5–5.5 mm in diametro, depresso-globosum, sulcis 5 latis, usque ad basem directis sulcatum.

Holotypus: MUÑIZ 15061; Cuba; Sierra de Escambray. Falda Norte del Pico Potrerillo, sobre diente de perro, cerca de la cuspide. Col.: O. MUÑIZ et Margarita FERNANDEZ, 8. Julio, 1984.

Obs.: *Coccothrinaci acunanae* León affinis quae a specie nostra foliis segmentis cca 44, usque ad 50 cm longis, floribus subsessilibus, fructibus non muricatis differt.

Palma de hasta 3–4 m de alto, con tronco cilindrico, erguido, de 6–10 cm de espesor*. Vaina de la hoja de 32–38 cm de largo y 11–15 cm de ancho, notablemente ansanchada hacia arriba, densamente entretrejida de fibras rígidas de 0.8–1.2 mm de grueso, la parte libre anchamente aovada o mayormente truncada, de 6–7 cm de largo, sin puntas libres. Pecíolo con la vaina de 75–80 cm de largo, la parte libre del pecíolo de 40–45 cm de largo, 1.5 cm de ancho debajo del ápice, convexo en ambas caras. Lígula 1, triangular-aovada, ligeramente 3-loba, de 1.5–2 cm de largo, aguda en el ápice. Limbo de la hoja orbicular o 3/4-orbicular, redondeado en la base, de 36–40 cm de largo y 40–42 cm de ancho; segmentos 38–42, los centrales de 36–38 cm de largo y 4.5–5 cm de ancho, connados en la base hasta una longitud de 12–14 cm, ligeramente atenuados arriba en una punta aguda de 8–9 cm de largo; los laterales lineal-subulados de 18–20 cm de largo y 6–8 mm de ancho, todos planos y verdes en el haz nervios primarios y secundarios visibles, no prominulos, terciarios a menudo obsoletos, azulado-verde pálidos en el envés, sin indumento plateado, densamente punteado por puntos amarillos prominulos, nervios primarios y secundarios poco prominulos, terciarios nulos.

Inflorescencias axilares encorvadas, compuestas de 2 racimos parciales largos, de 50–60 cm de largo con el pedunculo de 20–30 cm de largo, densamente bracteado; brácteas pergamáceas, lineal-aovadas o lineal-lanceoladas, de 12–15 cm de largo, muy poco tomentosas en el ápice, posteriormente glabras. Inflorescencias parciales muy ramosas, ramas laterales de hasta 30. Flores con pedicelos de 1–2 mm de largo, las bractéolas mas cortas que el pedicelo; lóbulos del periantio de 1 mm de largo, estrechamente triangular-subulados, mas estrechos que los filamentos en la base. Estambres 6–7, filamentos oblongo-trianguulares, brevemente connados en la base, mas largos que los lóbulos del periantio; anteras elíptico-oblongas o lineal-elípticas no aflechadas. Ovario anchamente aovado, diminutamente punteado, el estilo de igual largo que el ovario, ligeramente ensanchado hacia el ápice. Fruto deprimido globoso de 7–8 mm de diámetro; pericarpio papiráceo, muricado-punteado, rugoso. Semilla ósea, de 4.5–5.5 mm de diámetro, deprimido globosa, con surcos 5 bastante anchos dirigidos hasta a base.

Holotipo: MUÑIZ 15061; Cuba; Sierra de Escambray, Pico Potrerillo, Falda Norte, sobre diente de perro, cerca de la cuspide. Col.: O. MUÑIZ y Margarita FERNANDEZ. 8 Julio, 1984.

Afin a *Coccothrinax acunana* León (de la Sierra Maestra), que difiere de nuestra especie en tener hojas de cca 44 segmentos, de hasta 50 cm de largo, flores subsentadas y frutos no muricados.

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Mem. Soc. Cub. Hist. Nat. **13**: 107–156.
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PHYTOCHROME IN A HETEROPHYLLIC AQUATIC MACROPHYTE, *POTAMOGETON AMPLIFOLIUS* TUCKERM.

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Phytochrome was extracted from different organs of field-grown *Potamogeton amplifolius* Tuckerm., a heterophyllous aquatic macrophyte, using affinity chromatography on agarose-immobilized Cibacron Blue 3GA (AffiGel). The greatest amounts of undergraded phytochrome per gram freeze-dried tissue were obtained from roots/rhizomes and floating leaves. Purity of the final product varied with organ type and age of plant, suggesting that final amounts isolated may have been partially influenced by differences in protease levels and/or stability.

Introduction

Phytochrome functions as a major mediator in the regulation of many photomorphogenic effects in plants, including germination, stem growth and flowering. These responses are associated with phototransformation of the interconvertible forms of phytochrome, P_R and P_{FR} , in the regions of 660 and 730 nm respectively. Most of the available literature deals with photoresponses in terrestrial species, but such phenomena would be expected to be of great importance in aquatic plants in view of the variety of light intensity and quality which they experience during the course of their growth towards the water surface.

Members of the genus *Potamogeton* are known to have a positive light requirement for germination (SPENCE et al. 1971a), and in some species this process is reversibly responsive to red and far-red illumination (SPENCE et al. 1971b). Turion formation in *P. crispus* L. is influenced by light intensity and photoperiod (SASTROUTOMO 1980). In *Marsilea vestita* Hook. and Grev. it has been suggested that conversion of the water form to the land form may be affected by differences in light quality (GAUDET 1963). Although such observations suggest the involvement of phytochrome as a photoreceptor pigment, no attempts have yet been made to extract it from aquatic macrophytes.

Phytochrome was first obtained by BUTLER et al. (1959) from etiolated corn seedlings. Since that study, the majority of investigators have utilized etiolated seedlings because these contain significantly more phytochrome than does green tissue, and because chlorophyll interferes with the spectral measurement of this pigment (e.g. LANE et al. 1963, SIEGELMAN and BUTLER 1965, HOLMES and WAGNER 1980, SHIMAZAKI et al. 1981). LANE et al. (1963) were the first to detect phytochrome in partially purified extracts of green plants. TAYLOR and BONNER (1967) isolated phytochrome from a green alga and a liverwort. This pigment has also been found in green mosses (GILES and VON MALTZAHN 1968). SHIMAZAKI et al. (1981) obtained phytochrome from green pea tissues. The recent development of an affinity procedure utilizing agarose-immobilized Cibacron Blue 3GA (AffiGel) (SMITH and DANIELS 1981) has presented great potential for purification of phytochrome from tissues where this protein is present in low amounts.

The latter affinity procedure was utilized in the present study to isolate phytochrome from different organs of *Potamogeton amplifolius* Tuckerm. grown in the wild. This species is heterophyllous and is therefore exposed to the widest range of light conditions, and it has large leaves from which periphyton can be easily removed.

Materials and methods

Plants were obtained on 3 August 1982 and 28 June 1983 from a small, shallow pond on the western edge of the granitic Precambrian Shield, 5.1 km west of Rennie, Manitoba. A series of midday attenuation measurements of photosynthetically active radiation (400–700 nm) was made in 1983 using a Li-Cor Integrating Quantum Photometer with Underwater Quantum Sensor (Li-Cor Inc., Lincoln, Nebraska) (Fig. 1).

The plant material was collected by hand from a depth of 1 m. Plants harvested in 1982 had already set seed; those obtained in 1983 were only starting to flower and to unroll the floating leaves. The plants were washed and scraped as they were collected to remove periphyton. The different parts were separated and immediately placed in plastic bags on ice in lightproof containers. On return to the laboratory the material was frozen and subsequently freeze-dried a few weeks later prior to extraction.

Phytochrome was extracted from freeze-dried tissues using a modification of the procedure described by SMITH and DANIELS (1981). All steps were carried out at 0–5 °C under dim light from one General Electric green 25-watt bulb. Freeze-dried samples from 1982 were extracted in single 3–20 g lots, depending on amounts of material available; those from 1983 were each divided into 2 replicate lots of 6–40 g, except for flowers, which remained as one 11 g lot. Replicates were alternated so that the same plant parts were not extracted consecutively.

Each lot was pulse-homogenized in a blender with 200–600 ml of a solution containing 100 mM K-phosphate and 56 mM 2-mercaptoethanol, adjusted to pH 7.8 with conc. HCl. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at $7000 \times g$ for 25 min. The protein in the supernatant was precipitated by gradual addition of a concentrated ammonium sulphate solution such that the final concentration in the extract was 200 g/l. The sample was centrifuged at $6000 \times g$ for 20 min and the pellets were resuspended and

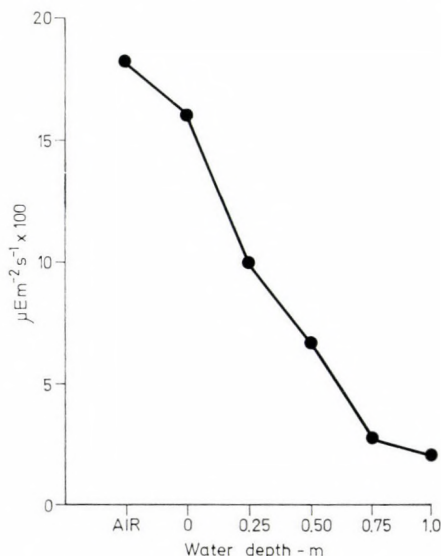


Fig. 1. Midday attenuation of photosynthetically active radiation at the study site

pooled in 50 ml of a solution containing 10 mM K-phosphate and 14 mM 2-mercaptoethanol, at pH 7.8 (10/14 KPM). This suspension was desalted on a 4×45 cm column of Sephadex G-50 (Pharmacia Fine Chemicals, Uppsala, Sweden) that had been equilibrated in 10/14 KPM. Fractions of 20 ml were collected. Those which appeared to contain phytochrome were pooled. Presence of phytochrome was monitored by measuring the change in absorbance at saturating irradiation at 667 vs. 730 nm.

The pooled fractions were combined with brushite (dibasic calcium phosphate) and allowed to stand overnight. Brushite was prepared not more than 1 day in advance by combining 150 ml of dry brushite with 400 ml of 0.6 M KCl, followed by 4 successive volume washes with 10/14 KPM.

The next day the brushite-extract mixture was batch eluted with 100 ml volumes of a solution containing 70 mM K-phosphate and 14 mM 2-mercaptoethanol, at pH 7.8. Elutriates were centrifuged at $5000 \times g$ for 5 min and monitored for phytochrome. Elution was continued until no further phytochrome activity was evident.

Brushite elutriates containing phytochrome were pooled and protein was precipitated by adding 61% by volume of a solution containing 435 g ammonium sulphate and 6 g Tris per liter (Ammono-Tris). The sample was centrifuged at $6000 \times g$ for 20 min. Pellets were resuspended in a total of 11–13 ml of a solution containing 100 mM K-phosphate and 14 mM 2-mercaptoethanol, at pH 7.8 (100/14 KPM). This suspension was applied to a 1×40 cm column of AffiGel Blue (100–200 mesh) (Bio-Rad Laboratories, Richmond, California) equilibrated in 100/14 KPM. The sample was followed by application of 5 ml of 0.5 M KCl in 100/14 KPM. The column was closed for 30 min. Afterwards it was washed with 4 column volumes of 0.5 M KCl in 100/14 KPM and with 1 column volume of 10 mM flavin mononucleotide in 100/14 KPM (FMN). The column was again closed for 30 min. Phytochrome was then eluted with FMN. Fractions of 10 ml were collected. Those absorbing at 667 nm were pooled and protein was precipitated with 82% by volume of Ammono-Tris. The sample was centrifuged at $10\,000 \times g$ for 20 min. Pellets were resuspended in a total volume of 5 ml of 100/14 KPM.

This sample was applied to a 2.5×45 cm column of Bio-Gel A-1.5 m (200–400 mesh) (Bio-Rad Laboratories) equilibrated in 100/14 KPM. Fractions of 5 ml were collected; those absorbing at 667 nm were pooled.

AffiGel was regenerated after each use by removing it from the column and washing it with 2 successive volumes of 8 M urea, then with 8 volumes of a solution containing 3 M KCl and 50 mM Tris, at pH 7.8 (SONG et al. 1981). The gel was repacked into the column and washed with 100/14 KPM. Sephadex and Bio-Gel were washed with their respective equilibrating buffers in the columns. Brushite was not reused.

Protein determinations were made using Coomassie Brilliant Blue G according to the method described by BRADFORD (1976).

Results and discussion

The amounts of protein present in crude extracts per gram of freeze-dried tissue varied in the different plant organs. In 1983 samples (Table 1) flowers and floating leaves showed the highest levels. A similar sequence appeared in 1982 samples, except that roots/rhizomes showed the highest levels and flowers were not examined as the plants had set seed. After the first ammonium sulphate precipitation, 20–25% of the original protein remained, a value comparable to that reported for this step by SMITH and DANIELS (1981) for etiolated rye seedlings.

Phytochrome eluted readily from brushite in 70 mM KPM buffer, and from AffiGel as a major peak with the first appearance of FMN in the eluate. A smaller subsequent peak probably represented degraded phytochrome subunits. For 1983 samples, floating leaves and roots/rhizomes showed approximately equal total areas under the elution profile curves per gram tissue,

Table 1

Initial protein and final A_{667} values per gram freeze-dried tissue for 1983 samples

| | Roots/ rhizomes | | Stems | | Submerged leaves | | Floating leaves | | Flowers |
|--|--------------------|------|-------|-----|---------------------|------|-----------------|------|---------|
| Crude protein, mg | 9.2, | 12.2 | 6.7, | 7.8 | 8.4, | 13.8 | 19.4, | 22.0 | 19.4 |
| Units $A_{667} \times 10^{-3}$ after Bio-Gel, per 1 ml total volume | 6.8, | 9.9 | 0.2, | 1.6 | 1.7, | 1.1 | 1.6, | 6.6 | 5.1 |

while all other organs yielded lesser amounts (Fig. 2). The total absorbance at 667 nm obtained from AffiGel for 1982 samples was higher than for 1983 lots for all organs except stems, which showed similar levels for both years. For 1982 samples, roots/rhizomes showed the greatest area under the elution profile curve ($6 \times$ that for 1983), followed by floating leaves (ca. $3 \times$ the 1983 value). The difference between submerged leaves and stems was much more pronounced in the 1982 samples.

Considerable variation has been reported for efficiency of recovery of phytochrome from AffiGel. SMITH and DANIELS (1981) reported a loss of only 36% of rye phytochrome between the brushite and AffiGel elution steps, while SONG et al. (1981) found that approximately 70% of oat phytochrome was lost on the AffiGel column. However in terms of protein, SMITH and DANIELS (1981) recovered roughly 8% of protein applied to AffiGel; this was similar to the value, on average, for this step in the present study.

In the Bio-Gel filtration step phytochrome eluted as a narrow peak within a broader protein band, but this was followed in some samples by another smaller peak, similar to that reported by SONG et al. (1981). According to the latter workers, the second peak represents a smaller degraded phytochrome unit. The results for 1983 samples, expressed as absorbance units at 667 nm per gram tissue (Table 1), showed the greatest values for roots/rhizomes, followed by floating leaves and flowers. Corresponding values for 1982 samples were: 24.5 for floating leaves, 7.5 for submerged leaves and 0.2 for stems. No value was obtained for roots/rhizomes at this step, but on the basis of the AffiGel results it would be expected to be greater than for floating leaves.

Losses at the Bio-Gel step, in terms of total absorbance at 667 nm, showed considerable variation, ranging from 45 to 80%. This is much higher than the ca. 35% loss reported at this step for rye phytochrome by SMITH and DANIELS (1981).

Amounts of phytochrome corresponding to the above absorbance values were estimated using the data of CORRELL et al. (1968) and SONG et al. (1981) for rye and oat seedling phytochrome respectively. For 1983 samples, this gave ca. (μg): roots/rhizomes 11, flowers 7, floating leaves 6, submerged leaves 2, and stems 1, recovered from Bio-Gel per gram freeze-dried tissue.

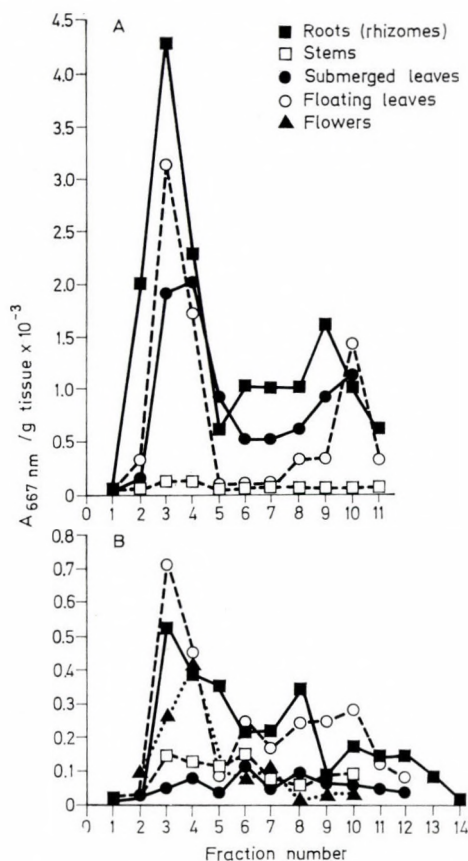


Fig. 2. AffiGel elution profiles for 1982 (A) and 1983 (B) samples. Fractions of 10 ml were collected. Values in A represent single samples; those in B are means of 2 replicates, except for flowers

For 1982 samples, ca. 30, 10 and 1 $\mu\text{g/g}$ were estimated for floating leaves, submerged leaves and stems respectively. For the most part, these values compare well with those in the literature. Using essentially the same purification technique, SMITH and DANIELS (1981) obtained close to 100 μg phytochrome per gram freeze-dried etiolated rye seedling tissue. Phytochrome content in green, light-grown plants has been reported to be about 1–2% of that in etiolated oats (HUNT and PRATT 1979) and 2–8% in peas (SHIMAZAKI et al. 1981). Thus the value of SMITH and DANIELS (1981) compares well with the values for the green tissues in the present study. However differences in relative content must be considered in relation to the amounts eluted from AffiGel (Fig. 2), because of differential recovery rates at the Bio-Gel step. Total amounts of phytochrome present in the crude extracts could not be measured because even the root-rhizome preparations contained some chlorophyll.

The phytochrome in the present study was obtained as P_R with an absorption peak at 667 nm. However extinction coefficients *per se* for phytochrome give little indication of the initial true concentration of this protein when some proteolytic degradation has occurred (BRIGGS and RICE 1972). In the present study homogeneity of the final product, as judged by the specific $A_{280} : A_{667}$ ratio, varied widely with sample type and age. For 1983 samples, a ratio of 1.0 was obtained only for roots/rhizomes. Floating leaves gave a less homogeneous product, with a ratio of ca. 10. Even greater values were seen for flowers, submerged leaves and stems. Products obtained from 1982 samples were more homogeneous, with values close to 1.0 for the two leaf types. In general, samples which gave the greatest A_{667} values also yielded the most homogeneous products. Thus phytochrome breakdown may have contributed towards some of the differences observed between organs and between samples of different age. It has been suggested by BRIGGS and RICE (1972) that differences in total phytochrome yield in different sample lots may be associated with differences in protease levels. Contaminating proteases may persist through a number of purification steps and affect the result. It is also possible that different forms of phytochrome may exist in different parts of the same plant (TAYLOR 1968) and these may differ in stability and yield.

Although phytochrome has been detected in all organs of higher plants, levels are generally highest in regions of the root and shoot where meristematic activity and development are taking place or have recently been completed (VINCE-PRUE 1975, KENDRICK and FRANKLAND 1976). The high values seen in roots/rhizomes and floating leaves in the present study were in line with this observation, but the 1982 material had already set seed and still appeared to maintain high levels in the floating leaves.

The floating leaves receive the greatest light intensities as well as the longest relative photoperiod, since attenuation increases rapidly with decreasing angle of incident radiation. At the study site the amount of radiation actually perceived by the submerged organs was likely much less than the values in Fig. 1 suggest, since the submerged surfaces were colonized by periphyton and there was shading within the plant stands. Differences in light intensity and photoperiod between underwater and surface regions have been among the factors that have been suggested to account for heterophyllic development in aquatic macrophytes (e.g. DAVIS 1962, BOSTRACK and MILLINGTON 1967). In *Potamogeton natans* L., floating leaves are not produced when white light intensities are low, or when plants are grown in red or green light, but appear at higher intensities of white light, or in blue light (e.g. SCULTHORPE 1967). It has been suggested by the latter worker and others that development of floating leaves may be correlated with light conditions through the effect of light on nutritional status of the plant. In some terrestrial species for

example, flowering induction appears to require photosynthesis or a supply of sucrose (VINCE-PRUE 1975).

In sunlight the R/FR ratio is approximately 1.3 (CUMMING 1963). This ratio decreases as the sun approaches the horizon (SHROPSHIRE 1973 in HOLMES and WAGNER 1980). Underwater ratios are always greater than the value in air because attenuation at 730 nm is more rapid than at 660 nm. In the photic zone this ratio may increase very rapidly with depth, particularly when little phytoplankton is present (SPENCE et al. 1971a). Thus as the macrophytes grow towards the surface, the shoot tips are exposed to progressively smaller R/FR ratios. This decrease may be among the factors which affect production of floating leaves and flowers. In *Hippuris vulgaris* L., low R/FR ratios during the appropriate photoperiod induce formation of aerial leaves on submerged shoots (BODKIN et al. 1980). Thus in heterophyllic species one of the functions of phytochrome in the leaves may be to gauge such ratios.

In summary affinity chromatography using agarose-immobilized Cibacron Blue 3GA has shown that phytochrome appears to be present in all organs of the heterophyllic macrophyte, *P. amplifolius*. The highest levels were found in roots/rhizomes and floating leaves. Higher values were associated with greater homogeneity of the final product, suggesting that less degradation had occurred in these samples. Because submerged aquatic macrophytes experience a wider range of R/FR values than do terrestrial species, phytochrome may be particularly important in the photomorphogenesis of heterophyllic aquatic plants.

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GROWTH, DNA-HISTONE RELATIONS AND IAA OXIDASE ACTIVITY OF NORMAL AND TUMOROUS TISSUE CULTURES REGULATED BY INDOLE DERIVATIVES, 2,4-D AND KINETIN

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Effect of indoleacetic acid (IAA), tryptophan, tryptamine, 5-hydroxy-tryptamine, 2,4-D and kinetin on growth, DNA and histone content as well as on IAA oxidase of tissue cultures of *Nicotiana glauca* (normal) and of tumor forming *N. glauca* × *N. langsdorffii* hybrid were studied. Effects of tryptophan derivatives and IAA are similar suggesting importance of the Tryptophan — Tryptamine — IAA biosynthetic pathway in the cultures studied. The hormonal treatments influence DNA, histone, IAA oxidase level and growth of the cultures. Effects of hormones depends on endogenous hormone and IAA oxidase concentration of tissues leading to a different sensitivity of normal and tumorous tissues. The histone — DNA relationships reflects the control of organization rather than that of growth and IAA oxidase formation.

Introduction

It has been demonstrated that tumorous tissues have higher metabolic and genetic activity than normal ones (BRAUN 1974, KOVÁCS 1967, 1971a, b, c, SMITH 1972, BEIDERBECK 1977). The specific metabolic and morphogenetic processes of tobacco tissues of normal and tumorous genotype are in close correlation with changes of histone to DNA ratio regulated by RNA level of cell. The isolated histone-DNA-RNA complex containing only tRNA suggests an important regulative role of tRNA in differentiation, morphogenesis and tumor formation (KOVÁCS 1971b, c, 1972, 1974).

It has also been demonstrated that growth, differentiation, organization and tumor formation are under hormonal control (SKOOG and MILLER 1957, SMITH 1972, KOVÁCS 1978, SHARP and EVANS 1981). Tissues of genetic tumors have an abnormally high level of growth substances (BUTCHER 1977, OOMS et al. 1981, SMITH 1972). In spite of the high hormone level of tumors an enhanced auxin destruction was also demonstrated (MEUDT 1970, KOVÁCS and MALIGA 1973).

In the presented experiments the less known role of the possible hormone precursors of indoleacetic acid as well as 2,4-D and kinetin in regulation of growth, hormone destruction and histone-DNA relations was studied.

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Materials and methods

Tissue cultures of *Nicotiana glauca* (normal) and of tumor forming F_1 hybrid of *N. glauca* \times *N. langsdorffii* (tumorous) were used as experimental materials. The normal cultures are unorganized calli while the tumorous ones have an increased shoot forming capacity requiring no growth hormones (auxins, cytokinins) for their growth (KOVÁCS 1967). The standard agar medium (KOVÁCS 1971) contained 0.2 mg/l kinetin and 10^{-7} , 10^{-6} , 10^{-5} and 10^{-4} M concentrations of each indoleacetic acid (IAA), tryptophan (TRY), tryptamine (TA), 5-hydroxy-tryptamine (serotonin, 5 OHTA), and 2,4-dichlorophenoxyacetic acid (2,4-D), or 2.0 mg/l IAA and 10^{-7} – 10^{-4} M of kinetin (KIN). The cultures were grown in the dark, at 26 °C.

DNA, histone content and indoleacetic acid oxidase (IAAO) enzyme activity were determined by the methods have used earlier (KOVÁCS 1971a, b, KOVÁCS and MALIGA 1973). One enzyme unit (EU) is 1 μ mole of destroyed IAA per min at 25 °C. Enzyme activity (EA) is expressed as EU per g fresh weight.

The data were subjected to statistical analysis.

Results

Fresh weight growth of the normal tissues was stimulated by IAA. This stimulation was proportional to increasing IAA concentrations (10^{-7} – 10^{-4} M, Fig. 1). A similar growth stimulation was brought about by TRY, TA, and 5 OHTA, too, except the moderate stimulation of 10^{-4} M concentration. Growth of the tumorous cultures was slightly influenced by IAA, TRY, TA and 5 OHTA treatments (10^{-4} M of the indole compounds used were somewhat inhibitory; Fig. 1).

Growth of the normal and tumorous cultures is stimulated by 10^{-7} M 2,4-D concentration. 10^{-6} M 2,4-D enhances fresh weight of normal tissues, only, while the higher concentrations of 2,4-D are inhibitory for growth of both the cultures (Fig. 1). KIN stimulates growth of the normal and tumorous cultures at 10^{-7} and 10^{-6} M concentrations. However, 10^{-5} and 10^{-4} M of KIN inhibit growth of both the normal and tumorous cultures (Fig. 1).

DNA content (μ g DNA per culture) of the normal cultures is increased by rising concentrations of each indole derivative studied. In the tumorous tissues treated with 10^{-7} – 10^{-5} M IAA, TA, TRY and 5 OHTA amount of DNA is rather similar however 10^{-4} M concentrations are somewhat inhibitory. Low levels of 2,4-D and KIN slightly enhance DNA content of both the normal and tumorous cultures. Higher levels of 2,4-D and KIN, however, reduce DNA content of the normal and tumorous tissues (Fig. 1).

Histone and DNA content of the cultures show parallel changes. The data indicate a proportional increase in histone content of normal cultures with increasing concentrations of indole derivatives. These treatments reduce amount of histones in the tumorous tissues (Fig. 1). 10^{-7} and 10^{-6} M 2,4-D slightly enhance histone level of normal cultures while 10^{-5} and 10^{-4} M concentrations reduce it. Histone content of the tumorous tissues is reduced by 10^{-6} – 10^{-4} M 2,4-D. KIN increases amount of histones in the normal tissues at 10^{-7} – 10^{-5} M concentrations. Histone level of tumorous cultures is rather similar at these KIN treatments (except 10^{-7} M; maximum). 10^{-4} M KIN strongly reduces histone content of both the tissues. DNA and histone content of tumorous cultures are higher than that of normal ones (Fig. 1).

The indole derivatives and 2,4-D increase histone to DNA ratio of normal tissues (10^{-4} M 5 OHTA causes a ratio like the untreated). In the tumorous cultures the ratios are almost similar at the same treatments. In the normal tissues only 10^{-7} and 10^{-6} M KIN cause some raise in histone to DNA ratio. Histone to DNA ratios of the tumorous tissues are increased by rising concentrations of KIN. In general, ratio of histone to DNA is higher in organizing tumorous cultures than that in unorganized normal calli (Fig. 1).

IAAO enzyme activity (EU per g fr. wt.) of the normal cultures is increased, in different degree, by each concentration of IAA, TRY, TA, 5 OHTA and 2,4-D (Fig. 1). In the

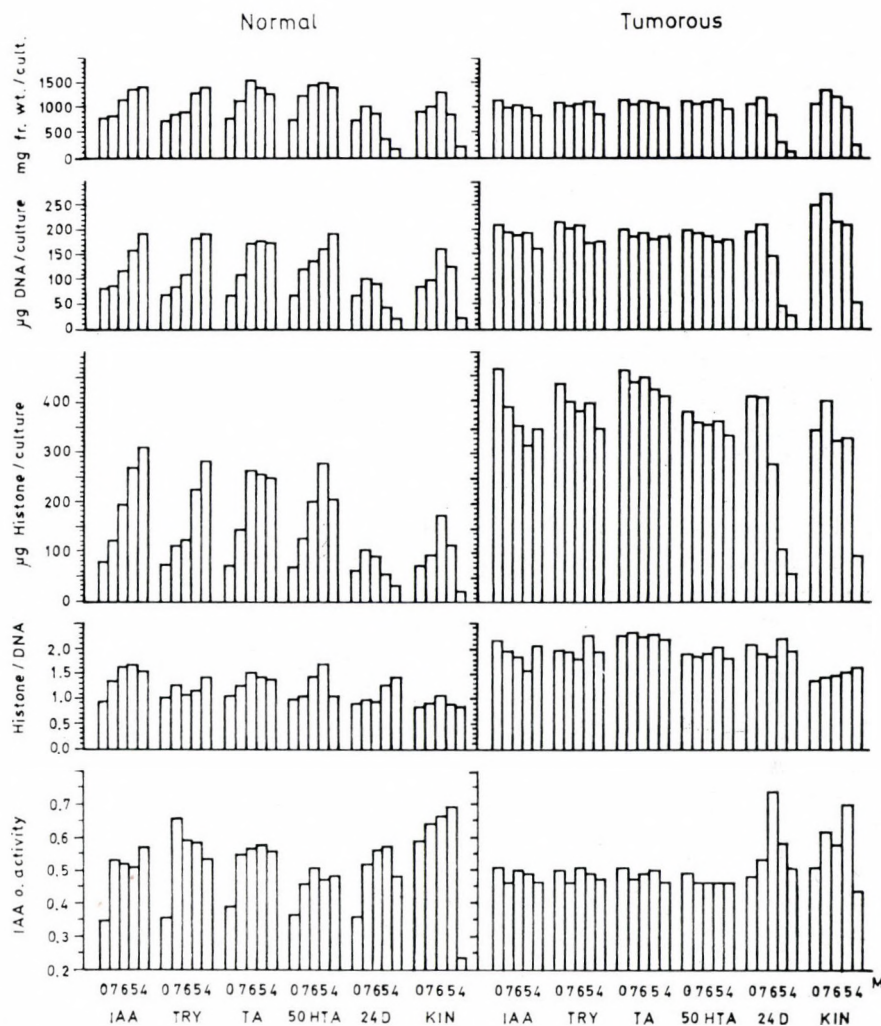


Fig. 1. Effect of indole derivatives, 2,4-D and kinetin on growth, DNA, histone content and IAAO enzyme activity of normal and tumorous tissue cultures

tumorous cultures the indole compounds are slightly inhibitory in comparison with untreated tissues. 2,4-D treatments stimulate IAAO activity (maximum: at 10^{-6} M) of both the cultures. 10^{-4} M KIN decreases IAAO activity of both the normal and tumorous cultures while its lower concentrations increase the enzyme activity (Fig. 1).

In the experiments sensitivity of the normal cultures against the indol derivatives used is higher than that of the tumorous ones. The IAA, TRY, TA and 5 OHTA treatments cause no significant changes in growth responses of tumorous tissues (insensitivity). Sensitivity of cultures appears to be depend on their IAAO activity. The data show no significant correlation between histone to DNA ratio and IAAO activity (Fig. 1).

Discussion

The experiments presented clearly show that effects of TRY, TA and 5 OHTA on normal and tumorous cultures are similar to that of IAA. TRY, TA and 5 OHTA influence growth, DNA, histone and IAAO content of the tissues as IAA does (cf. Fig. 1). Auxin (IAA) requirement of normal cultures can be satisfied by TRY, TA and 5 OHTA (and by the no precursor 2,4-D, too). These observations suggest presence and importance of TRY-TA-IAA biosynthetic pathway in normal and tumorous tobacco tissues. In other words, in biosynthesis of IAA of these cultures, as intermediers or precursors, TRY, TA and 5 OHTA can be included. These results are in accordance with the literary data (AUDUS 1973, WIDHOLM 1977).

According to the results presented in normal cultures both auxin and cytokinin are necessary to increase growth, DNA, histone and IAAO level (i.e. nucleic acid and protein synthesis). IAA, TRY, TA, 5 OHTA, 2,4-D or KIN alone are ineffective. This is valid for the tumorous tissues, too (KOVÁCS 1978), however, they do not require exogenous hormones for growth due to their high endogenous hormone (auxin, cytokinin) level (BEIDERBECK 1977, EINSET 1980). The high endogenous hormone level explains the relative ineffectiveness of the lower concentrations of the indole derivatives, 2,4-D and KIN treatments on tumorous tissues while high hormone levels are inhibitory. The high endogenous hormone level (especially that of cytokinins) of tumorous tissues keeps the high rate of their increased biosynthetic activities including IAAO synthesis (cf. Fig. 1; Kovács 1971b, c, 1978). The high IAAO activity of the tumorous cultures can also restrict effectiveness of the indole derivatives by destruction of the "new" IAA of exogene origin (indole derivatives) but cannot 2,4-D and KIN (cf. Kovács 1978). These conclusions and the results presented are in keeping with the general experiences (GASPAR et al. 1982). The different sensitivity of cultures of normal and tumorous genotype against the hormonal treatments confirms the existence of genotype-hormone interaction in tissues (Kovács 1978).

According to the results histone-DNA relationships reflect control of organizational conditions rather than that of growth or IAAO formation. The hormonal treatments can influence the histone-DNA relationships and IAAO activity (synthesis, too) in two different ways.

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REGULATION OF GROWTH CHARACTERISTICS AND ENZYME ACTIVITIES OF NORMAL AND TUMOROUS TISSUE CULTURES BY ACRIDINE ORANGE

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Growth characteristics (fresh weight, DNA, RNA, protein content), indoleacetic acid oxidase and amylase enzymes of tissue cultures of *Nicotiana glauca* and of tumor forming hybrid of *N. glauca* \times *N. langsdorffii* are influenced by acridine orange. The non-toxic concentrations of the dye can increase growth, nucleic acid and protein synthesis of tumorous cultures. The treatments are inhibitory for normal cultures. The dye stimulates amylase activity of tumorous cultures and indoleacetic acid oxidase activity of normal and tumorous cultures. IAA destruction of tumorous cultures can be inhibited by high level of the dye. There is a noticeable nucleic acid destruction in both cultures. Effects of acridine orange are mediated via influence of transcription and of endogenous indoleacetic acid level regulated by indoleacetic acid oxidase. Possible pathways of effects of acridine orange on growth, differentiation, organization, tumor formation and hormonal regulation are discussed.

Introduction

Numerous papers have been published on mutagenic, physiological (e.g. respiration, nucleic acid metabolism) and virological effects of acridine derivatives (NASIM and BRYCHCY 1979). However, little is known about effects of these compounds on growth, differentiation and organization in plants.

It has been described that AO inhibits sporulation in *Bacillus subtilis* (BURKE and SPIZIZEN 1977) and tumor formation of tumor prone tobacco hybrids as well as the characteristic shoot and bud formation of their tissue cultures (KOVÁCS 1970, KOVÁCS and PÁL 1976). The increased bud formation cannot be inhibited by actinomycin D (KOVÁCS 1970). An episomic explanation for origin of genetic tumors and increased organization of their tissue cultures has been suggested (KOVÁCS 1967, 1972). The acridine orange provides a useful tool to study genetic regulation of tumor formation and organization of cultures. The dye influences transcriptional and hormonal processes.

Materials and methods

In the experiments tissue cultures of normal tetraploid *Nicotiana glauca* (4x) and of tumor forming F₁ hybrid of *N. glauca* (4x) \times *N. langsdorffii* (2x) were used. The tissues were cultivated on an agar medium described previously (KOVÁCS 1971a; except: growth factor was 0.2 mg/l kinetin, only). Different concentrations of acridin orange (AO; 5, 50 and 500 μ M)

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were added to the medium. The tissue cultures were grown in the dark, at 26 °C. Two and three weeks old cultures were harvested for the determinations.

Nucleic acid and protein content as well as nucleic acid synthesis (measured by incorporation of ^{32}P into nucleic acids) of the cultures were determined by the methods described earlier (KOVÁCS 1971b).

Amylase enzyme activity was determined by method of JONES and VARNER (1967). 1 amylase enzyme unit is a change in optical density at 620 nm of 0.01 per 30 min at 37 °C. Indoleacetic acid oxidase (IAAO) activity was measured by the method used by KOVÁCS and MALIGA (1973). One enzyme unit (EÜ) of IAAO is 1 μg of destroyed IAA (indoleacetic acid) per min at 25 °C.

Possibility of disturbing effect of AO on quantitative determination of nucleic acids and proteins was also studied. In this experiments 0–1536 μg AO were added to the aliquots of tissue homogenate. (An aliquot of homogenate contained 1 g of tissue in 0.01 M phosphate buffer.) These samples containing AO were subjected to the above mentioned biochemical determinations. The results clearly showed that below 800 μg AO per g fresh weight tissue the dye had no disturbing effects. In tissue cultures treated with the highest AO concentration amount of AO was below 500 $\mu\text{g/g}$ fr.wt. leading to no disturbing effect in the determinations. (For measure AO content of tissues an ethanol extract of them was used to determine absorbance of the extract at 480 nm abs. max.)

Results

Fresh weight growth of the 2-week-old tumorous cultures were scarcely inhibited by 5 and 50 μM AO (Table 1). However, these 5 and 50 μM AO concentrations slightly increased the fresh weight of the 3-week-old tumorous cultures (by about 10%). 500 μM AO was inhibitory for growth (roughly 50%) of both 2 and 3 weeks old tumorous cultures (Table 1).

Table 1 shows that each AO concentration inhibits the fresh and dry weight growth of the 3-week-old normal callus cultures. Dry matter content of 2-week-old tumorous cultures is reduced by each AO treatment. Dry weight of 3-week-old tumorous cultures is enhanced by 5 and 50 μM AO while 500 μM reduces it. Per cent of dry material of normal and of tumorous cultures is increased by 500 μM AO, only, suggesting increased loss of water due to the toxic effect of this AO concentration (Table 1).

As parameters of growth changes level of DNA, RNA, proteins and indoleacetic acid oxidase as well as amylase enzyme activities of tissues were also studied.

Protein content of the 2-week-old tumorous tissues is diminished by AO treatments. In 3-week-old cultures of tumorous genotype 5 and 50 μM AO enhance level of protein content. Each concentration of AO decreases amount of proteins in normal cultures (Table 1).

Ratios of protein to RNA are similar in the normal and tumorous cultures (except 500 μM AO treatment) suggesting a similar protein turnover of the treated and untreated tissues. At 500 μM AO treatment the high ratios of protein to RNA are due to decreased level of RNA (Table 1 and 2).

Table 2 shows that 5 and 50 μM AO concentrations slightly increase DNA content of the 2-week-old tumorous cultures. AO treatments of 3-week-old tumorous cultures did not cause significant changes in their DNA content except 500 μM AO which reduced the amount of DNA by 52.6 per cent (Table 2). Incorporation of ^{32}P into DNA of these cultures was stimulated by 5 and 50 μM AO concentrations by 14.5 and 41.8 per cent, respectively (Table 2). (The discrepancy of the μg and cpm data suggest an increased degradation of DNA due to AO treatment.)

DNA level of normal tissues is decreased by each AO treatment. ^{32}P incorporation into DNA of these cultures is slightly stimulated by AO (maximum of 31% is at 50 μM AO concentration).

RNA content of 2-week-old tumorous cultures is decreased by 500 μM AO concentration only. In the 3-week-old tumorous cultures RNA content and ^{32}P incorporation into

RNA is enhanced by 5 and 50 μM AO concentrations suggesting a slightly increased RNA synthesis (Table 2). Toxic 500 μM AO concentration causes RNA degradation. In normal tissues RNA level and ^{32}P incorporation are reduced by each AO treatment (Table 2). The data suggest a strong inhibition of RNA synthesis by AO.

Table 1

Effect of AO on fresh, dry weight and protein content of normal and tumorous cultures

| Genotype | Age of tissue (week) | AO conc., μM | Fresh weight,* mg/cult. | Dry weight | | Protein, mg/culture | $\frac{\mu\text{g protein}}{\mu\text{g RNA}}$ |
|----------|----------------------|-------------------------|----------------------------|-----------------|------|------------------------|---|
| | | | | mg/cult. | % | | |
| Tumorous | 2 | 0 | 1142 \pm 81 | 55.3 \pm 1.58 | 4.84 | 6.29 \pm 1.44 | 5.34 |
| | | 5 | 1081 \pm 91 | 53.2 \pm 2.96 | 4.92 | 5.70 \pm 0.79 | 5.21 |
| | | 50 | 968 \pm 71 | 47.1 \pm 1.26 | 4.86 | 5.98 \pm 1.13 | 5.71 |
| | | 500 | 624 \pm 59 | 32.6 \pm 1.15 | 5.22 | 4.33 \pm 0.72 | 7.62 |
| | 3 | 0 | 1671 \pm 121 | 72.7 \pm 3.80 | 4.35 | 8.88 \pm 0.97 | 5.01 |
| | | 5 | 1826 \pm 139 | 76.0 \pm 3.66 | 4.16 | 10.03 \pm 0.89 | 5.38 |
| | | 50 | 1825 \pm 123 | 87.2 \pm 4.43 | 4.77 | 10.13 \pm 1.16 | 5.33 |
| | | 500 | 731 \pm 61 | 42.6 \pm 3.29 | 5.82 | 6.53 \pm 0.84 | 7.07 |
| | Normal | 0 | 716 \pm 43 | 31.6 \pm 1.38 | 4.41 | 2.58 \pm 0.32 | 5.41 |
| | | 5 | 652 \pm 33 | 27.6 \pm 1.53 | 4.23 | 2.20 \pm 0.19 | 5.30 |
| | | 50 | 462 \pm 29 | 25.4 \pm 2.61 | 5.49 | 1.65 \pm 0.11 | 5.63 |
| | | 500 | 205 \pm 12 | 12.5 \pm 1.67 | 6.09 | 1.53 \pm 0.13 | 7.92 |

* Initial weight: 150.5 \pm 5.68 mg/inoculum

Table 2

Effect of AO on nucleic acid content and on incorporation of ^{32}P into nucleic acids of normal and tumorous cultures

| Genotype | Age of cult. (week) | AO conc., μM | $\mu\text{g/culture}$ | | cpm/ μg | | RNA/DNA | |
|----------|---------------------|-------------------------|-----------------------|----------------|--------------------|----------------|---------------|------|
| | | | DNA | RNA | DNA | RNA | μg | cpm |
| Tumorous | 2 | 0 | 74 \pm 3 | 1178 \pm 129 | — | — | 15.92 | — |
| | | 5 | 80 \pm 2 | 1094 \pm 77 | — | — | 13.67 | — |
| | | 50 | 99 \pm 3 | 1046 \pm 101 | — | — | 10.56 | — |
| | | 500 | 70 \pm 2 | 568 \pm 35 | — | — | 8.11 | — |
| | 3 | 0 | 283 \pm 25 | 1773 \pm 89 | 123.3 \pm 11.4 | 404.0 \pm 54 | 6.26 | 3.27 |
| | | 5 | 247 \pm 16 | 1862 \pm 92 | 141.2 \pm 15.1 | 540.3 \pm 58 | 7.53 | 3.82 |
| | | 50 | 287 \pm 18 | 1898 \pm 129 | 174.9 \pm 19.3 | 552.6 \pm 22 | 6.61 | 3.16 |
| | | 500 | 146 \pm 13 | 923 \pm 60 | 126.7 \pm 18.0 | 478.1 \pm 28 | 6.32 | 3.77 |
| | Normal | 0 | 51 \pm 7 | 447 \pm 120 | 23.6 \pm 4.3 | 212.3 \pm 24 | 8.76 | 9.02 |
| | | 5 | 34 \pm 4 | 415 \pm 81 | 25.5 \pm 2.5 | 203.9 \pm 14 | 12.20 | 7.99 |
| | | 50 | 24 \pm 5 | 293 \pm 83 | 31.1 \pm 4.5 | 178.4 \pm 14 | 12.20 | 5.74 |
| | | 500 | 19 \pm 1 | 193 \pm 65 | 25.9 \pm 5.4 | 105.2 \pm 11 | 10.15 | 4.06 |

Table 3
Effect of AO on IAA oxidase and amylase enzyme activity of normal and tumorous tissue cultures

| Genotype | Age of tissues (week) | AO conc., μM | Enzyme activities | | | |
|----------|-----------------------|-------------------------|-------------------|-------------|------------------|-------------|
| | | | IAAO | | Amylase | |
| | | | EU/culture | EU/mg prot. | EU/culture | EU/mg prot. |
| Tumorous | 2 | 0 | — | — | 66.5 \pm 9.2 | 10.57 |
| | | 5 | — | — | 62.0 \pm 9.1 | 10.87 |
| | | 50 | — | — | 52.7 \pm 8.1 | 8.81 |
| | | 500 | — | — | 31.1 \pm 5.1 | 7.18 |
| | 3 | 0 | 39.3 \pm 7.3 | 4.43 | 267.3 \pm 26.6 | 30.10 |
| | | 5 | 47.0 \pm 8.0 | 4.70 | 322.0 \pm 19.3 | 32.10 |
| | | 50 | 33.3 \pm 7.5 | 3.28 | 325.3 \pm 21.9 | 32.11 |
| | | 500 | 22.1 \pm 3.4 | 3.38 | 159.7 \pm 10.3 | 24.45 |
| | Normal | 0 | 9.9 \pm 2.5 | 3.83 | T* | T |
| | | 5 | 12.2 \pm 2.7 | 5.54 | T | T |
| | | 50 | 12.7 \pm 3.3 | 7.69 | T | T |
| | | 500 | 2.3 \pm 0.4 | 1.50 | T | T |

T* = traces

Reduction of ratio of RNA to DNA in 2-week-old tumorous cultures is mainly due to decrease of RNA synthesis. This ratio practically unchanged in the 3-week-old cultures. In the normal cultures the ratio of RNA to DNA is mainly reduced due to inhibition of RNA synthesis (Table 2).

Alpha amylase enzyme activity is present in traces in the normal cultures. Activity of this enzyme is inhibited by the AO treatments in 2-week-old tumorous tissues (Table 3). Alpha amylase activity of 3-week-old cultures is stimulated by 5 and 50 μM AO and it is inhibited by 500 μM treatment.

IAAO enzyme activity of tumorous tissues was stimulated by 5 μM AO while other concentrations were inhibitory (Table 3). In the normal cultures both 5 and 50 μM AO concentrations increased IAAO activity (with a maximum at 50 μM). 500 μM AO was inhibitory for the enzyme activity (Table 3). There is a correlation between fresh weight growth and IAAO activity of cultures (see Tables 1 and 3). A high IAAO activity is accompanied by inhibition of growth and vice versa.

Discussion

The experiments presented in this paper indicate that effects of AO treatments on growth characteristics of the tissue cultures depend on their genotype (genotype-AO interaction). The hormone dependent normal callus cultures have higher sensitivity against AO than the hormone independent

genetic tumorous cultures having high organ forming capacity. AO inhibits growth characteristics of normal cultures much more than those of the tumorous ones. The recessive sensitivity of the normal glauca genome is not manifested in the tumorous hybrid cells.

Growth and morphogenesis of tissue cultures are regulated by the IAA oxidase enzyme system (KOVÁCS 1977, 1979a, b, MEHTA 1980). IAAO activity of the tumorous tissues is rather inhibited by AO treatment leading to growth stimulation. In the normal tissues AO increases the IAAO activity explaining their decreased growth. At the same time amylase enzyme activity is stimulated by AO in the tumorous cultures (except of 2-week old tissues). The experiments presented suggest a differential transcription of the genes coding these enzymes regulated by AO treatments.

Changes of protein and RNA content in the AO treated normal and tumorous cultures are similar (cf. Tables 1, 2) suggesting no translation is affected by AO. According to the data presented AO treatment can rather influence processes of transcription. This conclusion is also proved by the differential effect of AO on RNA synthesis of normal and tumorous tissues. DNA synthesis of both cultures is slightly influenced by AO. The RNA synthesis is inhibited by the dye, only in the normal cultures.

On the basis of intercalating and binding ability of AO to DNA (SCHREIBER and DANNE 1974; NASIM and BRYCHCY 1979) the dye can directly influence the differential transcription of genes coding specific proteins of morphogenesis by its different affinity to specific DNA regions. On the other hand, AO can inhibit RNA polymerases (MATTICK and NAGLEY 1977).

According to Table 2 the effect of AO treatments on RNA level depends on DNA content of tissues suggesting the importance of direct binding of AO to DNA in regulation of transcription. However, the differential effect of AO on IAAO and amylase level of cells may emphasize role of regulation of selective transcription by RNA polymerases having different affinity to AO. The multiple forms of DNA-dependent RNA polymerases of eukaryotic cells (TRAVERS 1976, WARNER and VERSTEEGH 1974) may have different transcriptional specificity and activity.

The strong inhibitory effect of 500 μ M AO is due to inhibition of transcription involving binding of the dye to DNA and to RNA polymerases, respectively. Effects of the lower AO concentrations on the cultures are mainly mediated by hormonal (endogenous IAA) influences (cf. relationship between IAAO activity and fresh weight growth). Thus, AO affects selective transcription of genes coding IAA oxidase enzyme system bringing about changes in amount of endogenous IAA of cells. The growth, morphogenetic processes and level of some enzymes of the cultures are influenced by the direct effect of AO on transcription and also by the changed endogenous hormone level influencing the transcription of the specific genes, too. The genotype-AO

interaction is based on different DNA content of normal and tumorous cells. Transcription of genes depends on ratio of AO to DNA (i.e. on free template surface).

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GROWTH RESPONSE OF PLANT CALLUS TISSUE TO TOXIC HEAVY METAL COMPOUNDS AND -IONS CONTAMINATING THE ENVIRONMENT

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Secondary tobacco (*Nicotiana tabacum* L.) callus tissues were tested for the effect of more frequently occurring toxic heavy metal compounds contaminating the environment. The compounds and their bivalent cations, respectively, included in the experiment were: cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), zinc (Zn). The concentrations used were: 10^{-4} – 10^{-6} , except mercury of which they were 10^{-5} – 10^{-7} M. The callus tissues were incubated on MURASHIGE and SKOOG (1962) culture medium for 4 weeks. The hormone supplements of the culture medium were: IES, Kin and GA_3 . Incubation took place at $25 (\pm 2)^\circ\text{C}$, in 16-hour light and 8-hour dark periods under an illumination of 120 W/m^2 . To control the effect of the metal ions the fresh weight and percentage drymatter content of the tissues were measured, and the number of cells per unit weight (g) counted.

As shown by the data of analyses the heavy metal compounds tested — with the exception of Cd — inhibited the increase of fresh weight in the isolated callus tissue of tobacco even at the lowest concentration used (10^{-6} – 10^{-7} M; as for the metal: 0.02–0.2 mg/l). The rate of inhibition grew with the increase of concentration; the highest concentrations even caused loss of water or lethality to the cells. With an increase in the concentrations the percentage drymatter content of the tissues grew, and its growth showed the opposite tendency to the increase of fresh weight. This may be related with the low water- and cytoplasm-, and relatively high wall-material contents of cells. The number of cells in a unit weight (g) of callus tissue generally grew with the increasing concentrations of compounds in comparison to the control, which suggests the inhibition of the expansion of cells. The metal compounds tested in our experiments inhibited the growth of the isolated plant tissues even at relatively low concentrations, and their toxic effects contribute new data to confirming the danger of environment contamination.

Introduction

Our environment is being increasingly contaminated by the heavy metal scraps (DÄSSLER 1979, KOVÁCS 1975). Some of them — even at very low concentrations — may have a toxic effect on the cells of plants that serve for human or animal food, and when accumulated on the human organism as well (KOVÁCS 1981, PAIS et al. 1977). The distribution, concentration and toxic effect of scrap-metals present in the soil or air have so far been examined mainly from the point of view of uptake by higher plants and accumulation in their organs (WALLACE et al. 1977a, 1977b, 1977c). Some sporadic data concerning the lethal effects on plants of certain heavy metal ions are also available (BONALY et al. 1980, DIJKSHOORN et al. 1979, FILIPPIS et al. 1981, WALLACE et al. 1977a, 1977b). However, if their toxic (growth inhibitory, necrotic, lethal) action mechanisms are to be better understood, a closer analysis of the lesions of tissues composing the plant organs seems to be necessary. Thus, to determine the limit of tolerance shown by the cells to heavy metal compounds we carried out infor-

matory experiments with those metal ions known for toxicity as most frequently occurring in the surroundings of plants, with concentration series of cadmium (Cd)-, copper (Cu)-, mercury (Hg)-, nickel (Ni)-, lead (Pb)- and zinc (Zn)-compounds. For the determination of the limit of tolerance of cells isolated tobacco callus tissue was used on the basis of earlier experiments (MARÓTI and BOGNÁR 1980, MARÓTI et al. 1981, MARÓTI et al. 1983, TÓTH and MARÓTI 1979).

Material and method

The heavy metals indicated were placed in the MURASHIGE and SKOOG (1962) culture medium in the form of the following compounds: $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2 \text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, HgCl_2 , $\text{NiSO}_4 \cdot 7 \text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$. The concentrations used ranged between 10^{-7} and 10^{-4} M.

Apart from the mineral macroelements the basic culture medium only contained $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ (8.6 mg/l) and $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ (0.025 mg/l) of all the metal ions to be tested, and was completed with 2.0 mg glycine, 0.1 mg thiamine-HCl, 0.5 mg nicotinic acid amide, 0.5 mg pyridoxin-HCl, 100.0 mg inositol, 2.0 mg indole acetic acid (IAA), 0.2 mg kinetin (Kin) and 1.0 mg gibberellic acid (GA) per litre. The concentration of agar was 1 per cent, the value of pH 5.7 before autoclaving (at 112 °C for 30 min).

The effect exercised on the tissues by the heavy metal compounds used was tested on callus tissues isolated from the stem of tobacco (*Nicotiana tabacum* L.). The cultures were secondary cultures of steady growth, isolated several decades earlier, consisting of unorganized cell populations. In flasks containing 25 ml culture medium tissue inocula — 200 mg for each — were placed and incubated for four weeks. The cultures grew at a temperature of $25 (\pm 2)$ °C, under illumination conditions of 16-hour light and 8-hour dark period. Illumination was supplied with Orion 20 W F.7 Daylight and Tungsram 20 W F.30 White type fluorescent lamps (120 W/m²).

The measured data of the variants are averages of 10 tissue samples (flasks) per each, for which the deviation was also determined. To check up on the effects of the compounds used we measured the initial and final fresh weights and percentage drymatter contents of the tissues, and counted the number of cells in a g unit weight. We calculated the daily rate of weight increase for the cultures and the so-called relative increase, which shows the multiplication of weight of the original inoculum. The cell numbers were determined by means of a Bürker chamber after the tissues had been macerated in chromic acid (shaken in 4% chromic acid at 70 °C for 2–15 minutes) (THOMAS and DAVEY 1975). The measured and calculated data have been tabulated and expressed as percentages to the control.

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Results and discussion

The results of experiments are summarized in Tables 1 to 6, while the weights and cell numbers relative to the control are shown in Figs 1 to 6.

As to the effect exercised on the callus tissue of tobacco the six metal ions tested have the common feature that each of them acts first of all on the increase of fresh weight, the percentage drymatter content and the number of cells as a function of concentration. It was also a general observation that with an increase in the concentrations of the metal ions the fresh weights of the tissues decreased, while the percentage drymatter contents and the num-

Table 1
Effect of cadmium on the growth of tobacco callus tissue

| Hormones, mg/l | Cd(CH ₃ COO) ₂ , mg/l M | Fresh weight | | Dry weight | | Number of cells | | Daily weight increase | | Relative growth |
|-------------------------------|---|-----------------|-----------------|------------|-----------------|----------------------|-----------------|-----------------------|-----------------|--------------------|
| | | g/flask | % of control | % | % of control | 10 ³ ×n/g | % of control | mg | % of control | |
| IAA Kin GA ₃ | 26.6 | \bar{x} 0.150 | 3 | 1.28 | 203 | 3758 | 168 | — | — | — |
| | 10 ⁻⁴ | $\pm s$ 0.030 | | 0.09 | | 359 | | | | |
| | 13.3 | \bar{x} 1.133 | 24 | 0.76 | 120 | 2620 | 117 | 33.32 | 21 | 4.66 |
| | 5×10 ⁻⁵ | $\pm s$ 0.375 | | 0.01 | | 275 | | | | |
| | 2.6 | \bar{x} 8.824 | 191 | 0.54 | 86 | 2720 | 121 | 347.39 | 220 | 48.63 |
| | 10 ⁻⁵ | $\pm s$ 3.324 | | 0.03 | | 180 | | | | |
| | 1.3 | \bar{x} 8.991 | 195 | 0.53 | 84 | 2470 | 110 | 313.96 | 199 | 43.95 |
| | 5×10 ⁻⁶ | $\pm s$ 3.070 | | 0.12 | | 259 | | | | |
| | 0.26 | \bar{x} 8.677 | 187 | 0.57 | 90 | 2754 | 123 | 302.75 | 192 | 42.38 |
| | 10 ⁻⁶ | $\pm s$ 3.980 | | 0.04 | | 205 | | | | |
| Control | | \bar{x} 4.618 | 100 | 0.63 | 100 | 2238 | 100 | 157.78 | 100 | 22.09 |
| | | $\pm s$ 1.176 | | 0.10 | | 284 | | | | |

Table 2
Effect of copper on the growth of tobacco callus tissue

| Hormones, mg/l | CuSO ₄ , mg/l M | Fresh weight | | Dry weight | | Number of cells | | Daily weight increase | | Relative growth |
|-------------------------------|----------------------------------|-----------------|-----------------|------------|-----------------|----------------------|-----------------|-----------------------|-----------------|--------------------|
| | | g/flask | % of control | % | % of control | 10 ³ ×n/g | % of control | mg | % of control | |
| IAA Kin GA ₃ | 24.9 | \bar{x} 0.114 | 2 | 0.91 | 147 | 4130 | 185 | — | — | — |
| | 10 ⁻⁴ | $\pm s$ 0.013 | | 0.08 | | 41 | | | | |
| | 12.4 | \bar{x} 0.150 | 3 | 1.02 | 162 | 3419 | 153 | — | — | — |
| | 5×10 ⁻⁵ | $\pm s$ 0.022 | | 0.07 | | 33 | | | | |
| | 2.5 | \bar{x} 3.667 | 79 | 0.66 | 105 | 3215 | 144 | 123.82 | 78 | 17.33 |
| | 10 ⁻⁵ | $\pm s$ 0.630 | | 0.01 | | 25 | | | | |
| | 1.2 | \bar{x} 3.796 | 82 | 0.57 | 90 | 2319 | 104 | 128.42 | 81 | 17.98 |
| | 5×10 ⁻⁶ | $\pm s$ 0.632 | | 0.04 | | 15 | | | | |
| | 0.25 | \bar{x} 4.390 | 95 | 0.63 | 100 | 2239 | 100 | 149.64 | 95 | 20.95 |
| | 10 ⁻⁶ | $\pm s$ 0.942 | | 0.06 | | 9 | | | | |
| Control | | \bar{x} 4.618 | 100 | 0.63 | 100 | 2238 | 100 | 157.78 | 100 | 22.09 |
| | | $\pm s$ 1.176 | | 0.10 | | 284 | | | | |

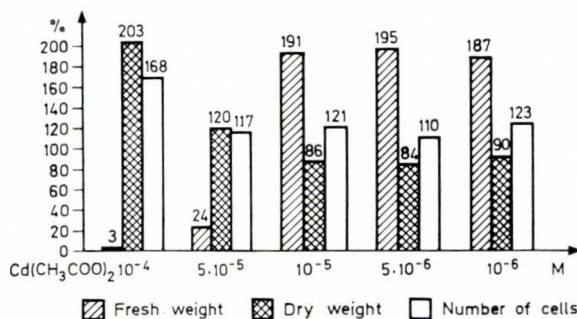


Fig. 1. Effects of various concentrations of cadmium acetate and cadmium ion, respectively, on tobacco callus tissues, as a percentage to the control

ber of cells per unit weight (g) increased. On the basis of fresh weight analyses the six compounds tested can be placed in three groups. In the first group only the high concentrations — of cadmium e.g. — inhibit the weight increase, the lower ones stimulate it (Table 1, Fig. 1). The second group includes those heavy metals which at high concentrations not only inhibit the weight increase but may even cause lethality, while when present in small amounts the fresh weight of the callus may be nearly equal to that of the control. Such are the copper, the mercury, the nickel (Tables 2 to 4, Figs 2 to 4). The third group — lead and zinc — inhibits at all concentrations used the growth of tissue that reaches 25 per cent of that in the control at the most (Tables 5, 6, Figs 5, 6).

Table 3

Effect of mercury on the growth of tobacco callus tissue

| Hormones, mg/l | HgCl ₂ , mg/l M | Fresh weight | | Dry weight | | Number of cells | | Daily weight increase | | Relative growth |
|---|----------------------------------|-----------------|-----------------|------------|-----------------|----------------------|-----------------|-----------------------|-----------------|--------------------|
| | | g/flask | % of control | % | % of control | 10 ³ ×n/g | % of control | mg | % of control | |
| IAA 2.0 Kin 0.2 GA ₃ 1.0 | 2.70 | \bar{x} 0.099 | 2 | 1.04 | 165 | 2127 | 95 | — | — | — |
| | 10 ⁻⁵ | $\pm s$ 0.011 | | 0.51 | | 92 | | | | |
| | 1.35 | \bar{x} 1.469 | 31 | 0.83 | 131 | 2376 | 106 | 45.32 | 29 | 6.34 |
| | 5 × 10 ⁻⁶ | $\pm s$ 0.565 | | 0.11 | | 60 | | | | |
| | 0.27 | \bar{x} 1.654 | 36 | 0.85 | 135 | 2509 | 112 | 51.92 | 33 | 7.27 |
| | 10 ⁻⁶ | $\pm s$ 0.036 | | 0.12 | | 195 | | | | |
| | 0.13 | \bar{x} 1.845 | 40 | 0.40 | 63 | 2415 | 108 | 58.75 | 37 | 8.42 |
| | 5 × 10 ⁻⁷ | $\pm s$ 0.725 | | 0.20 | | 794 | | | | |
| | 0.03 | \bar{x} 3.165 | 68 | 0.57 | 90 | 2656 | 119 | 105.89 | 67 | 14.82 |
| | 10 ⁻⁷ | $\pm s$ 1.089 | | 0.01 | | 1207 | | | | |
| Control | | \bar{x} 4.618 | 100 | 0.63 | 100 | 2238 | 100 | 157.78 | 100 | 22.09 |
| | | $\pm s$ 1.176 | | 0.10 | | 284 | | | | |

Table 4

Effect of nickel on the growth of tobacco callus tissue

| Hormones, mg/l | NiSO ₄ , mg/l M | Fresh weight | | Dry weight | | Number of cells | | Daily weight increase | | Relative growth |
|---|----------------------------------|-----------------|-----------------|------------|-----------------|----------------------|-----------------|-----------------------|-----------------|--------------------|
| | | g/flask | % of control | % | % of control | 10 ³ ×n/g | % of control | mg | % of control | |
| IAA 2.0 Kin 0.2 GA ₃ 1.0 | 28.0 | \bar{x} 0.153 | 3 | 0.89 | 141 | 2508 | 112 | — | — | — |
| | 10 ⁻⁴ | $\pm s$ 0.008 | | 0.03 | | 2 | | | | |
| | 14.0 | \bar{x} 0.195 | 4 | 0.96 | 152 | 2451 | 109 | — | — | — |
| | 5 × 10 ⁻⁵ | $\pm s$ 0.031 | | 0.30 | | 2 | | | | |
| | 2.8 | \bar{x} 0.346 | 7 | 1.08 | 158 | 2726 | 122 | 5.21 | 3 | 0.73 |
| | 10 ⁻⁵ | $\pm s$ 0.109 | | 0.05 | | 11 | | | | |
| | 1.4 | \bar{x} 1.244 | 27 | 0.83 | 131 | 2220 | 99 | 37.28 | 24 | 5.22 |
| | 5 × 10 ⁻⁶ | $\pm s$ 0.549 | | 0.02 | | 2 | | | | |
| | 0.28 | \bar{x} 4.297 | 93 | 0.65 | 103 | 2824 | 126 | 146.32 | 93 | 20.48 |
| | 10 ⁻⁶ | $\pm s$ 1.168 | | 0.24 | | 2 | | | | |
| Control | | \bar{x} 4.618 | 100 | 0.63 | 100 | 2238 | 100 | 157.78 | 100 | 22.09 |
| | | $\pm s$ 1.176 | | 0.10 | | 284 | | | | |

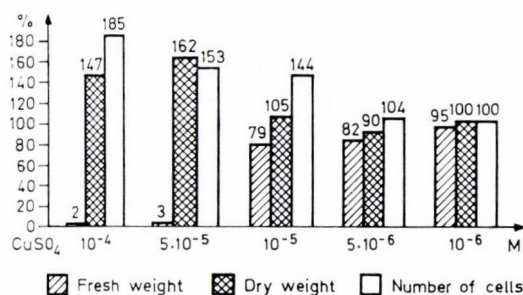


Fig. 2. Effects of various concentrations of copper sulphate and copper ion, respectively, on tobacco callus tissues, as a percentage to the control

Table 5

Effect of lead on the growth of tobacco callus tissue

| Hormones, mg/l | Pb(NO ₃) ₂ , mg/l M | Fresh weight | | Dry weight | | Number of cells | | Daily weight increase | | Relative growth |
|---|--|-----------------|--------------|------------|--------------|----------------------|--------------|-----------------------|--------------|-----------------|
| | | g/flask | % of control | % | % of control | 10 ³ ×n/g | % of control | mg | % of control | |
| IAA 2.0 Kin 0.2 GA ₃ 1.0 | 29.9 | \bar{x} 0.269 | 6 | 1.10 | 174 | 3469 | 155 | 2.46 | 2 | 0.34 |
| | 10 ⁻⁴ | $\pm s$ 0.038 | | 0.65 | | 49 | | | | |
| | 14.5 | \bar{x} 0.591 | 13 | 0.85 | 135 | 2752 | 123 | 13.96 | 9 | 1.95 |
| | 5×10 ⁻⁵ | $\pm s$ 0.104 | | 0.05 | | 26 | | | | |
| | 2.9 | \bar{x} 1.062 | 23 | 0.80 | 127 | 2354 | 105 | 30.78 | 19 | 4.31 |
| | 10 ⁻⁵ | $\pm s$ 0.220 | | 0.08 | | 16 | | | | |
| | 1.45 | \bar{x} 1.146 | 25 | 0.72 | 114 | 2692 | 120 | 33.78 | 21 | 4.73 |
| | 5×10 ⁻⁶ | $\pm s$ 0.752 | | 0.08 | | 20 | | | | |
| | 0.29 | \bar{x} 1.146 | 25 | 0.66 | 104 | 2683 | 119 | 33.78 | 21 | 4.73 |
| | 10 ⁻⁶ | $\pm s$ 0.036 | | 0.05 | | 13 | | | | |
| Control | | \bar{x} 4.618 | 100 | 0.63 | 100 | 2238 | 100 | 157.78 | 100 | 22.09 |
| | | $\pm s$ 1.176 | | 0.10 | | 284 | | | | |

Table 6

Effect of zinc on the growth of tobacco callus tissue

| Hormones, mg/l | ZnSO ₄ , mg/l M | Fresh weight | | Dry weight | | Number of cells | | Daily weight increase | | Relative growth |
|---|----------------------------------|-----------------|--------------|------------|--------------|----------------------|--------------|-----------------------|--------------|-----------------|
| | | g/flask | % of control | % | % of control | 10 ³ ×n/g | % of control | mg | % of control | |
| IAA 2.0 Kin 0.2 GA ₃ 1.0 | 28.7 | \bar{x} 0.616 | 13 | 1.00 | 158 | 2210 | 99 | 14.85 | 9 | 2.08 |
| | 10 ⁻⁴ | $\pm s$ 0.200 | | 0.08 | | 21 | | | | |
| | 14.3 | \bar{x} 0.888 | 20 | 0.90 | 142 | 2193 | 98 | 24.28 | 15 | 3.40 |
| | 5×10 ⁻⁵ | $\pm s$ 0.121 | | 0.01 | | 12 | | | | |
| | 2.8 | \bar{x} 0.782 | 17 | 1.04 | 165 | 2483 | 111 | 20.78 | 13 | 2.91 |
| | 10 ⁻⁵ | $\pm s$ 0.289 | | 0.05 | | 6 | | | | |
| | 1.41 | \bar{x} 0.897 | 19 | 0.86 | 136 | 3004 | 134 | 24.89 | 16 | 3.48 |
| | 5×10 ⁻⁶ | $\pm s$ 0.269 | | 0.12 | | 88 | | | | |
| | 0.28 | \bar{x} 1.163 | 25 | 0.73 | 115 | 2710 | 121 | 34.39 | 22 | 4.81 |
| | 10 ⁻⁶ | $\pm s$ 0.574 | | 0.38 | | 13 | | | | |
| Control | | \bar{x} 4.618 | 100 | 0.63 | 100 | 2238 | 100 | 157.78 | 100 | 22.09 |
| | | $\pm s$ 1.176 | | 0.10 | | 284 | | | | |

Examination for dry weight revealed that the cadmium, copper and mercury increased the percentage of dry weight (by 5–103 per cent) only when present at higher concentrations (10^{-4} – 10^{-6} M) (Tables 1 to 3, Figs 1 to 3), while the nickel, lead and zinc increased it at every concentration used (by 3–74 per cent) (Tables 4 to 6, Figs 4 to 6).

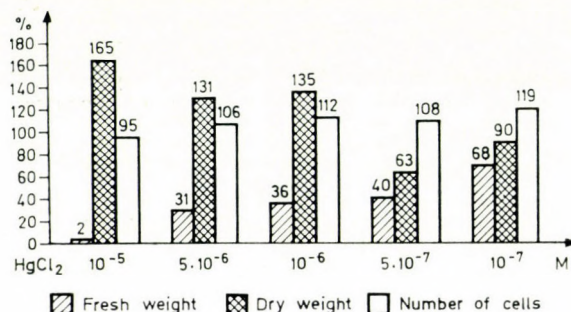


Fig. 3. Effects of various concentrations of mercury chloride and mercury metal ion, respectively, on tobacco callus tissues, as a percentage to the control

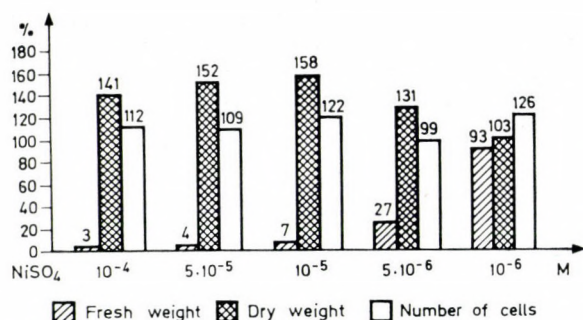


Fig. 4. Effects of various concentrations of nickel sulphate and nickel metal ion, respectively, on tobacco callus tissues, as a percentage to the control

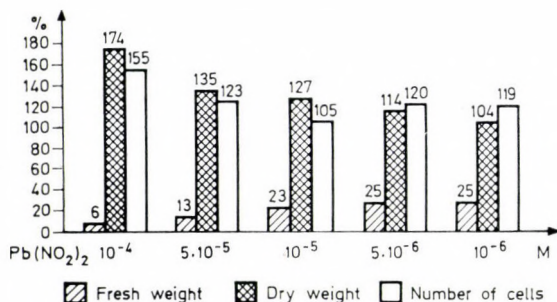


Fig. 5. Effects of various concentrations of lead nitrite and lead metal ion, respectively, on tobacco callus tissues, as a percentage to the control

As seen from the number of cells per g callus tissues, all compounds tested increased, in general, the cell number. There were, however, differences between the effects of the individual metal ions, especially at high concentrations. The compounds of Cd, Cu, Pb increased the number of cells compared to the control by 55–85 per cent e.g. at a concentration of 10^{-4} M, while the

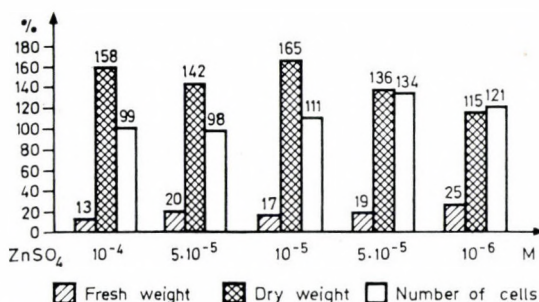


Fig. 6. Effects of various concentrations of zinc sulphate and zinc metal ion, respectively, on tobacco callus tissues, as a percentage to the control

same concentrations of Hg-, Ni- and Zn-compounds resulted in a cell number nearly equal to that of the control. This suggests differences in their action in mechanism of cell division (Tables 1 to 6, Figs 1 to 6).

A many-sided examination of the action of toxic metal ions contaminating our environment on the plants is today particularly justified (DÄSSLER 1979, KOVÁCS 1975, PAIS 1980, WALLACE 1977, WALLACE et al. 1977c). It is also clear, that in addition to studies on the relation between environment and intact plant, an analysis of the mechanism of action exercised by the more frequently occurring toxic compounds on the cell- and tissue structure and metabolism of the plant is indispensable (BONALY et al. 1980, DIJKSHOORN et al. 1979, FILIPPIS et al. 1981). With this in view we tested the effect of some heavy metal compounds on plant callus tissues too. According to our experiences of several years of analyses of the above cultures under the described conditions the growth parameters of the control tissue cultures (fresh weight, percentage dry weight and cell number) show realistic values (MARÓTI and BOGNÁR 1980, MARÓTI et al. 1981, 1983). They can therefore serve as a suitable basis of comparison for determining the effects of metal compounds. The concentrations of the metal compounds used in the experiments were chosen partly on the basis of their actual concentration in the environment, partly after literary data (DIJKSHOORN et al. 1979, FILIPPIS et al. 1981, PAIS 1980, WALLACE et al. 1977c).

The effect of the metal compounds tested on the increase of fresh weight is best seen from the data of daily increase compared to the control, and above

all from the relative growth data. In the course of 4 weeks the weight increased some 22-fold in the control. Cadmium causes growth inhibition or lethality only at high concentrations, while its lower concentrations have a stimulatory effect, as pointed out by a number of authors (BONALY et al. 1980, WEIGEL and JÄGER 1980a). The growth inhibition caused by the other metal compounds tested (those of Cu, Hg, Ni, Pb, Zn) is generally confirmed by the data of literature too. Growth inhibition by lead and zinc is of very great extent, while mercury has a considerably inhibitory effect even at 10^{-7} M (DIJKSHOORN et al. 1979, NAG et al. 1981).

The percentage dry weight generally shows an increasing tendency, opposed to the inhibition of fresh weight by metal ions. This phenomenon can be explained, in general, with the water loss of the cells and the relative accumulation of the material of cell-wall, as also found in our earlier experiments (MARÓTI et al. 1981, 1983). The number of cells per unit weight was the largest in the case of the lowest fresh weight in response to almost every compound tested, therefore the inhibition of the weight increase can supposedly traced back to the prevention of the expansion rather than of the division of cells. This hypothesis is more or less supported by the trend of the percentage dry weight.

There are, though, data available on the action mechanism of inhibition by toxic heavy metals, they come, however, mostly from examinations of intact higher plants or of alga cells. It is known e.g. that of the compounds tested Cd, Hg and Zn at certain concentrations have a lethal effect on the cells of the alga *Euglena* (FILIPPIS et al. 1981), Cd, Cu, Ni, Pb and Zn are generally toxic to plants (DIJKSHOORN et al. 1979, WALLACE et al. 1977a, 1977b), Ni, Pb and Zn hinder the development of fruit (PATEL et al. 1977, WALLACE et al. 1977a, 1977b), Cd, Hg and Zn inhibit the division and motion of cells in *Euglena* (FILIPPIS et al. 1981), Cd, Cu, Hg and Zn check the activity of numerous enzymes (NAG et al. 1981, WEIGEL and JÄGER 1980a, b), Cu, Hg and Zn have a toxic effect on the synthesis of deoxyribonucleic acid in the nucleus (BONALY et al. 1980) and prevent the formation of chlorophyll (NAG et al. 1981). Having been obtained with intact plants or in experiments with their cells these data can be applied to isolated callus tissue only with a proper revaluation. So much can, however, be established from our experiment data that the heavy metal compounds used for testing — except cadmium — inhibited the fresh weight increase of tissues at all concentrations, and — in opposition to it — the percentage drymatter content and the number of cells per unit weight increased, the biochemical mechanism of which is to be cleared up by further investigations. However, the stimulatory effect of low concentrations of cadmium acetate cannot be satisfactorily interpreted either from the relevant literary data or from our analyses (WALLACE et al. 1977d).

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EFFECT OF LIGHT AND TEMPERATURE ON LEAF MORPHOLOGY AND PRODUCTIVITY IN *DIGITALIS LANATA* EHRH.

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Digitalis lanata Ehrh. plants propagated by tissue culture method were examined under controlled conditions in Conviron E 15 and G 30 type phytochambers. The processes of adaptation under three different light intensities — 8 klx, 16 klx and 32 klx — simulating low and warm temperated vegetation periods were studied as well. The average area of one leaf (from 10.6 to 39.1 cm²) and total leaf area of individual plant (from 728.2 to 1145.6 cm²) were increased as a result of compensation for decreasing light intensity. Rearrangement of the leaf anatomical structure (by reducing the ratio of palisade parenchyma) was the other form of adaptation. The maximum dry-mass was produced under 32 klx light intensity (10.6 g · plant⁻¹). On the other hand the lanatoside C production was determined by interaction of dry-matter accumulation and the change of the glycoside concentration: the maximum value (33.6 mg · plant⁻¹) was measured at 16 klx. The air temperature had less effectiveness as that of the light intensity — under low temperature conditions both of the dry-matter and C-glycoside production decreased comparing to the value of the given light intensity.

Keywords: *Digitalis lanata*, light effect, temperature effect, morphology, dry-matter production, cardiac glycosides, lanatoside-C concentration and production.

Introduction

Digitalis lanata Ehrh., a member of the Scrophulariaceae family has been known since the early thirties as a species containing cardiac glycosides (MEYER 1973). Its natural occurrence is restricted to the Pannonian and Balkanic flora provinces, and production of the leaf drug for industry became possible only by cultivation of the species. One of the main objectives ever since the first growing experiments has been to disclose the influence of environmental factors. BALBAA et al. (1974) studied the role of spacing, MILLETTI and FRENGUELLI (1975) the effect of the nutrient status, BALBAA et al. (1971) the interaction of water and nutrient supply. Other authors tried to clarify the effect of complex system of factors, as e.g. NYÁRÁDI-SZABADY and PÁL (1981) compared two extreme habitats. However, studies on the role of light and temperature are relatively few. Through observations in cultivation experiments (KUTIÁK 1977) arrived at the conclusion that an increase in the number of sunshine hours and proportion of UV radiation proved to be favourable for the growth and development of plants. FORMANOWICZOWA and KOZŁOWSKI (1971) detected the positive effect of light treatment on germination. However, experiments to study the role of light and temperature under controlled conditions have not been carried out so far.

Thus, in our experiments the objective of determining the role of light and temperature was set under exact system of conditions. Being close connection between primary and secondary metabolic processes (BELL and CHARLWOOD 1980) the glycoside formation and accumulation can be interpreted by the change of primary processes as well.

Materials and methods

Plant material

The experiments were carried out with the *Digitalis lanata* Ehrh. cv. 'Oxford' grown in Hungary. To ensure the homogeneity the plants after two years inbreeding were propagated by tissue culture method (DOBOS *et al.* 1982). The treatments were started after two-week incubation measuring the reaction of 90 individuals in every light variations.

Standard growth conditions

The experiment were set up in (Conviron) E 15 and a modified G 30 type phyto-chambers of Canadian make. The plants were grown in a perlite-sand mixture medium of 1 : 1 ratio. After a two-week period of incubation the water capacity of the culture medium was adjusted to a 70 per cent value of saturation, maintained by distilled water every two or three days. During the incubation period the relative humidity of air was 80 per cent, then subsequently 60 per cent in daytime and 70 per cent at night throughout the experiments.

Nutrient conditions

The nutrients were supplied by KNOP and HOUGLAND solutions once a week. The doses of major elements given per culture pot were: 6.0 mg N, 1.2 mg P and 6.3 mg K. From the 145th day the doses were doubled.

Temperature conditions

During the incubation period the air temperature was kept steadily at 20 °C. After, low and warm conditions were simulated taking into consideration the average data of 80 years of Budapest and the vegetation period of the species:

| | Warm conditions, °C (day/night) | Low t. conditions, °C (day/night) |
|----------------|------------------------------------|--------------------------------------|
| 1st– 5th week | 16.0/10.0 | 16.0/10.0 |
| 6th– 9th week | 18.5/11.5 | 18.5/11.5 |
| 10th–14th week | 21.0/12.0 | 19.0/12.0 |
| 15th–19th week | 26.0/16.0 | 20.0/12.0 |
| 20th–24th week | 22.0/13.0 | 19.0/12.0 |
| 25th–28th week | 18.5/11.5 | 18.5/11.5 |
| 29th– | 16.0/10.0 | 16.0/10.0 |

Light conditions

Light intensity was provided uniformly by General Electric F72T12/CW/VHO fluorescent tubes completed with incandescent lamps to 11 per cent of the energy. In the incubation period the light intensity was 4 klx. Subsequently, in a daily 14-hour rhythm the following light intensities were applied:

- 8 klx (in warm condition system)
- 16 klx (in warm and low condition system)
- 32 klx (in warm condition system)

Morphological and production analysis

The growth of plants was continuously registered. The number of leaf per plant and the size of leaf was measured and at the same time the anatomical changes of leaves were studied. The leaves were fixed in FAA (JENSEN 1962), washed in distilled water and frozen by a 1 : 2 mixture of gum-arabic and water (with carbon dioxide) onto a freezing block. 25 µm transverse-sections made by cryostat were stained by 0.2 per cent solution of toluidine-blue. For chelate formation a 1 : 1 ratio of 2 per cent potassium iodide and 2 per cent potassium ferro-cyanide solution were used (ROMHÁNYI 1968).

At the age of 210 days leaf number per plant, average leaf size, leaf-, and root production and C-glycoside content were measured. The glycoside content was measured by using the modified methods of SZILÁGYI *et al.* (1977) and HORVÁTH (1982).

Results

Morphological observations

Even in the initial phase of growth visible morphological differentiation occurred as an effect of different light intensities and temperatures. In high light intensity and at low temperature too, characteristically small leaves were formed, while at low light intensity thin and large leaves developed (Table 1).

The data of mean length of leaves and changes of leaf area show that under poor illuminations (8 klx) the plant tries to compensate the deficiency of light by increasing its assimilation surface. This fact is shown by average area of one leaf grown nearly fourfold (from 10.6 to 39.1 cm²) under 8 klx illumination. The temperature somewhat modifies this process, slightly decreasing leaf area of the given light intensity. The total leaf area of one plant changes in an analogous manner.

Parallel with the increase of the leaf area the thinning of the leaf-blade occurred. The 472 μ m thickness of the leaf-blade measured at 32 klx is reduced to 385 μ m at 16 klx and to 338 μ m at 8 klx. The cold temperature program seems to play a secondary role in this respect, too. The change of thickness is connected with anatomical modifications, as it can be seen in Fig. 1. In the case of high light intensities the palisade parenchyma, while at low light intensity the spongy parenchyma is dominant. The ratio of two parenchyma under deficient light condition is 0.44, while at 32 klx intensity 3.57.

Dry-matter production

The light and temperature dependence of dry-matter production is clearly indicated by the average number of leaf per plant (Fig. 2). The largest number of leaves developed at high 32 klx light intensity — twice as many as with an illumination of 8 klx. There were no considerable differences in respect of temperature.

Table 1

Effect of light and temperature on morphology and anatomy of Digitalis lanata leaves

| Treatment | Morphology | | | Transverse section of leaves | | | |
|--------------------|----------------------------|---------------------|---------------------------|------------------------------|--------|-------|------------------------------------|
| | Mean length of leaves (cm) | Surface of one leaf | Leaf surface of one plant | Thickness | | | Ratio of pal. and spon. parenchyma |
| | | | | palisade | spongy | total | |
| | | (cm ²) | (μ m) | | | | |
| “Warm temperature” | | | | | | | |
| 8 klx | 24.2 | 39.1 | 1145.6 | 76 | 174 | 338 | 0.44 |
| 16 klx | 20.2 | 15.0 | 783.0 | 189 | 138 | 385 | 1.37 |
| 32 klx | 16.9 | 10.6 | 728.2 | 243 | 68 | 472 | 3.57 |
| “Low temperature” | | | | | | | |
| 16 klx | 16.3 | 13.3 | 728.8 | 212 | 139 | 399 | 1.52 |
| SD _{0.1%} | 1.2 | 7.9 | 10.7 | 24 | 13 | 21 | 0.62 |

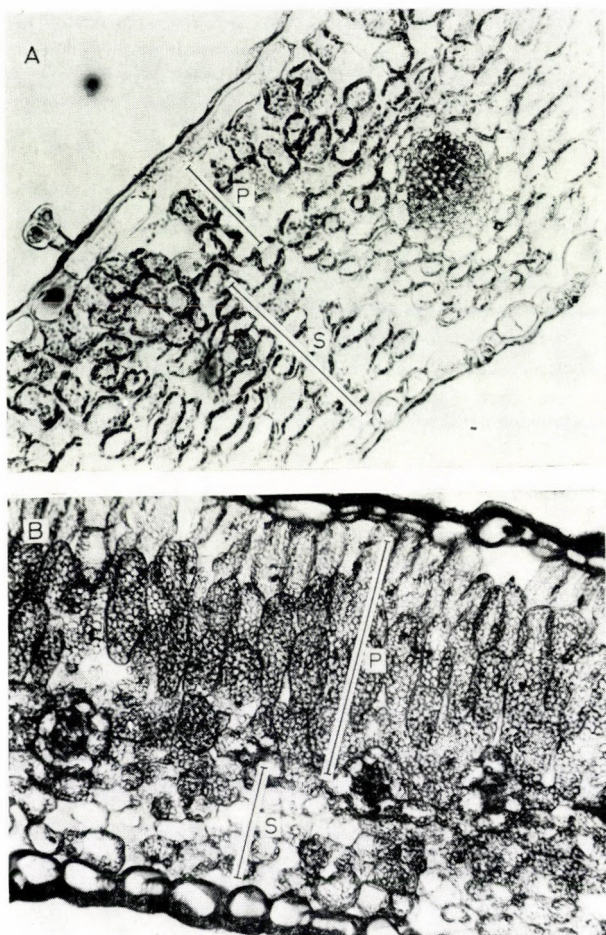


Fig. 1. A, transverse section of the leaf blade affected by low (8 klx) illumination. The presence of the spongy parenchyma (s) is dominant. The ratio of palisade parenchyma (p) remained below zero. B, transverse section of leaf blade grown under high (32 klx) illumination. The thickness of the leaf is increased by about 30 per cent and the formation of a characteristic palisade (p) layer is prevalent. The mean ratio value of palisade-spongy parenchyma is as much as 3.57

The total plant production: the amounts of leaf and root and their ratio show a characteristic trend (Fig. 3). At low light intensity (8 klx) two-third of the total amount of dry-matter accumulates in the leaves. At the medium light intensity the dry-matter production of the root exceeds even in absolute value that of the leaf. At 32 klx illumination production and ratio of root increases further on.

According to the results (Table 2) 7.1 g dry-matter could be produced by one plant at 8 klx, which increases to 9.3 g at 16 klx and to 10.6 g at 32 klx intensity. The intensification of primary processes is also shown by the fact that the dry-matter, photosynthetized by a unit leaf area, grows more than twofold parallelly. As an effect of temperature cold condition reduces production characteristic of the given light intensity by about 20 per cent.

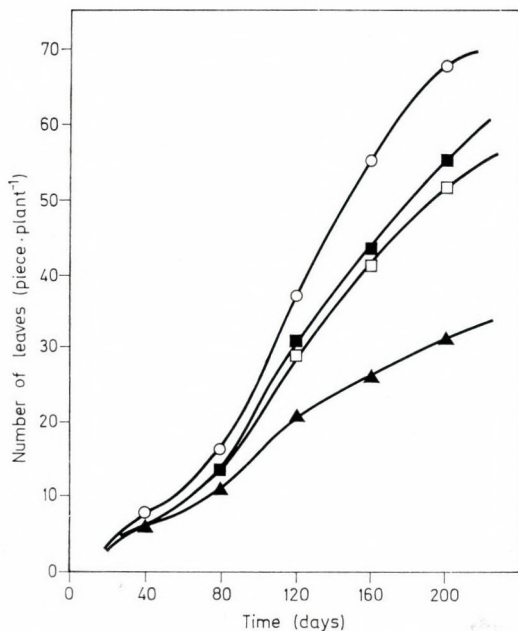


Fig. 2. Changes of leaf number per plant affected by 8 klx (▲), 16 klx (■) and 32 klx (○) illumination and by low temperature conditions (□)

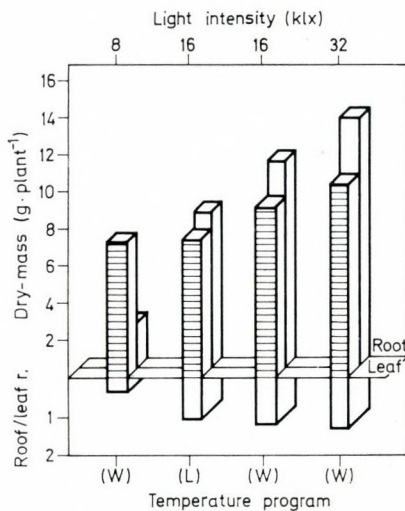


Fig. 3. Changes of dry-matter production of individual plants affected by light intensity (8 klx, 16 klx, 32 klx) and warm (W) and low (L) temperature conditions. The overall dry-matter production is promoted by light, while the absolute and relative amount of root (Root/leaf r.) shows an increase of a higher degree

Table 2

Effect of light and temperature on dry-mass and lanatoside-C production of Digitalis lanata

| Treatment | Leaf production (plant ⁻¹) | | | C-glycoside production (plant ⁻¹) | | |
|---------------------|--|-------------|--|---|------------|---|
| | Number of leaves (piece) | dry wt. (g) | dry wt. per surface (mg · cm ⁻²) | Concn. (mg · g ⁻¹) | Total (mg) | Calculated pro surface (mg · cm ⁻²) |
| "Warm temperature" | | | | | | |
| 8 klx | 29.3 | 7.1 | 6.2 | 4.4 | 31.4 | 0.0271 |
| 16 klx | 52.2 | 9.3 | 12.2 | 3.6 | 33.6 | 0.0442 |
| 32 klx | 68.7 | 10.6 | 15.0 | 2.7 | 28.6 | 0.0404 |
| "Low temperature" | | | | | | |
| 16 klx | 54.8 | 7.2 | 10.2 | 4.1 | 29.1 | 0.0414 |
| LSD _{0.1%} | 13.8 | 2.4 | 4.5 | — | 2.3 | 0.0051 |

Glycoside production

The lanatoside-C content calculated on unit dry-matter (glycoside concentration) reaches its maximum at low light intensity, even if the difference is not significant matematically. In responses to low temperature some increase in the lanatoside-C content can be observed, too. However there will be considerable differences when the total lanatoside C production is evaluated. The change by increasing the dry-matter production parallel to the light intensity and a simultaneous lowering of the lanatoside-C concentration could be expressed by an orthogonal polynomial

$$Y = 120.2 - 38.9 X + 5.7 X^2 - 0.3 X^3$$

Consequently, the glycoside production per plant has its maximum at about a light intensity of 16 klx. This is indicated, by the C-glycoside production per unit leaf area too, which reaches its maximum value — 0.0442 mg · cm⁻² — at 16 klx.

Discussion

With the widening knowledge about secondary plant products it becomes more and more obvious, that the term "secondary", suggesting the less important role of these compounds is hardly acceptable any longer. The same opinion is supported by the recent summarizing work of MOTHES (1980). An increasing number of evidences of the ecological role of secondary products have been found (HARBORNE 1977). These data are related first of all with the adaptation to the ecosystem (pollination, animal toxins, allelopathy, etc.). Less evidence is discovered about the relationship of primary-secondary processes and about the role of secondary compounds in the ecological adaptation of individuals. So, in the case of *Digitalis lanata* it was not clear enough what differences the primary adaptation changes caused in the formation and accumulation of cardiac glycosides in response to some environmental effect.

According to our investigations the light intensity (and to a lesser extent the cold and warm conditions) induce easy to follow adaptation processes in the primary production. The plant tries to compensate for the lower light intensity by increasing its average leaf area, and the total photosynthetizing surface. The high degree of adaptation is indicated by the fact that the increase of average leaf area is fourfold and the total leaf area is nearly twofold. The tissue structure shows a parallel modification too.

The leaf structure characteristic of field conditions develops at the 16 klx intensity of light (Ph.Hg.V. 1954). The palisade parenchyma consists of 3–4 cell rows, while the spongy parenchyma is of close set isodiametric cells. This leaf structure is modified by low light intensities in order to make better use of the light. The leaf-blade becomes thinner, the cells of the palisade parenchyma are square or radially slightly elongated, while those of the spongy parenchyma tangentially elongate (Fig. 1).

The primary processes characterized by dry-matter accumulation reflect the above changes of adaptation. The other way of adaptation to poor light is the accumulation of the dry-matter in the leaf, while the ratio of the root remains much below. However, adaptation to higher light intensity is shown by the intensification of unit leaf area production. It reaches its maximum (150 mg · cm⁻²) unambiguously at 32 klx.

The secondary production characterized by the change of the lanatoside-C content follows a complex adaptation response. Here distinction must be made between the lanatoside-C concentration and the total lanatoside-C production of the plant. Namely, parallel to the increase of light intensity the lanatoside-C concentration (mg · g⁻¹, dry-matter) shows a slight decrease. The total lanatoside-C production is, however, determined by the total productivity of the plant too. The production peak is measured at a medium light intensity (16 klx) when the intensity of dry-matter accumulation is fairly high, and that of glycoside accumulation is still but slightly reduced. In response to low air temperatures a decrease in the total lanatoside-C production can be observed due to the reduced dry-matter production.

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EFFECT OF THE SPECTRAL COMPOSITION OF LIGHT ON THE PHYTOMASS PRODUCTION OF LOESS-SWARD, IN RELATION TO THE CHLOROPHYLL CONTENT

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The effect of the spectral composition of light on the net aboveground phytomass production and the chlorophyll content of dominant species in stands of natural composition of four subassociations of the *Salvio-Festucetum rupicolae pannonicum* Zólyomi (1958) loess-sward plant association was studied in photostat under controlled conditions. Three kinds of light treatment were used: the plant stands were illuminated by fluorescent lamps with blue, red and compound "white" light, respectively, of identical energy content ($60 \text{ W} \cdot \text{m}^{-2}$) for 12 hours a day over 120 days.

The aboveground drymatter production and energy consumption of the plants significantly varied with the spectral composition of light. In the whole period of examination the highest net production was obtained in red light ($6.7032 \text{ g} \cdot \text{dm}^{-2}$) and the lowest in blue light ($2.3466 \text{ g} \cdot \text{dm}^{-2}$) on the average; with plants grown in compound "white" light the dry-matter production (mean value in 120 days: $3.6132 \text{ g} \cdot \text{dm}^{-2}$) was closer to that attained in blue light. The energy utilization relative to the total radiation during the whole period of examination was 3.72 per cent with the red, 1.30 per cent with the blue and 2.01 per cent with the compound "white" light.

Changes in the spectral composition of light did not cause essential differences in the average chlorophyll content of the species. For the dominant species a very close positive linear regression correlation between the amount of aboveground phytobiomass and its chlorophyll content on dry weight basis was pointed out in each light treatment. Our results prove the dependence of the production and energy utilization of the vegetation on the number of photons that the chlorophyll molecules are able to absorb.

Introduction

The effect of the spectral composition of light on the intensity of carbon dioxide assimilation, the primary organic matter production and on the products of photosynthesis, respectively, has so far been studied only in certain plant species. Results of experiments with algae were published e.g. by HAUSCHILD, NELSON and KROTKOV (1961, 1964), KOWALLIK (1962), PIRSON and KOWALLIK (1964). Most of the authors studied the photosynthetic production of angiospermous plants (VOSKRESENSKAYA and GRISHINA 1959, KWACK and DUNN 1961, TREGUNNA, KROTKOV and NELSON 1962, HORVÁTH 1965, STEVENSON and DUNN 1965, KOLTAY and HORVÁTH 1967, SZÁSZ 1967, KADMAN-ZAHAVI and ALVAREZ-VEGA 1968, WARRINGTON and MITCHELL 1976, HORVÁTH, MIHALIK and TAKÁCS 1978, HORVÁTH and MIHALIK 1980, and others). Many summarizing evaluations have also been published in the subject, e.g. by VEEN and MELJER (1959), KLESCHNIN (1960), NUERNBERGK (1961), VOSKRESENSKAYA (1965, 1972) and HORVÁTH, MIHALIK and TAKÁCS (1980). We attempted to find out the effect of the spectral composition of light on the net primary production in stands of natural composition of a plant association, under controlled conditions.

We studied the effects of various spectral compositions on the chlorophyll content in the dominant species, and searched for a correlation between the chlorophyll content and the amount of aboveground phytobiomass. The relationship between net production and chlorophyll content has been studied by a number of authors in terrestrial plant associations too [see e.g. the comprehensive work of FEKETE (1972)]. On the correlation in a loess-sward vegetation we too have published data (ENDRÉDI and HORVÁTH 1977).

The experiment under discussion was carried out with stands of four subassociations of a loess-sward plant association (*Salvio-Festucetum rupicolae pannonicum* Zólyomi 1958) previously studied first in the field then under controlled conditions, using compound "white" light of $120 \text{ W} \cdot \text{m}^{-2}$ energy content (ENDRÉDI and HORVÁTH 1976, 1977, ENDRÉDI 1984). Of the results account was given in two lectures (ENDRÉDI and HORVÁTH 1978, ENDRÉDI 1981).

Present paper shows the trend of net aboveground drymatter production and energy consumption in response to different light treatments, with the chlorophyll contents of the dominant species taken into consideration. Data on the photosynthetic pigment contents of the species will be given in detail in another paper.

Material and method

The loess-sward subassociation studied were (Soó 1964, 1973, 1980):

1. *Salvio-Festucetum rupicolae pannonicum* Zólyomi 1958 *festucetosum rupicolae*;
2. *Salvio-Festucetum rupicolae pannonicum* Zólyomi 1958 *stipetosum capillatae* Soó 1959;
3. *Salvio-Festucetum rupicolae pannonicum* Zólyomi 1958 *andropogonetosum* (Boros 1953) Soó 1959;
4. *Salvio-Festucetum rupicolae pannonicum* Zólyomi 1958 *poëtiosum angustifoliae* Zólyomi 1959.

The stands come from the loess-sward vegetation of the western slope of Nagyhegy at Dunaföldvár (ENDRÉDI and HORVÁTH 1976). We cut $20 \times 20 \times 20$ cm sods out of the plant stand and placed them in a photostat. In the photostat the daily rhythm of temperature was $18\text{--}28^\circ\text{C}$, the relative humidity of air ranged between 45 and 80 per cent, and the carbon dioxide content corresponded to the natural concentration (0.033 per cent). The amount of water supplied to the plants in the form of distilled water every day was nearly the same throughout the experiment.

Three kinds of light treatment were applied. The plant stands were illuminated with blue, red and as a control compound "white" light by means of 40 W Tungsrham type F-blue-, F-red- and F₃₃ fluorescent lamps for 12 hours a day. The energy content of the light was the same, about $60 \text{ W} \cdot \text{m}^{-2}$, with all three spectral compositions.

At the beginning of the experiment the plant stands were cut back to the ground level. It was then that the species composition and cover of the vegetation were established. Of the samples taken from each subassociation those were placed in the photostat for which the species composition and cover were found to be practically the same, whereby the possibility of comparing them for drymatter production—by the fraction too—obtained with the different light treatments was ensured.

The effect of the spectral composition of light was studied in three experiment series representing the replications, first in 1978, then in 1981 and 1982, under perfectly identical conditions.

The plant stands were raised from 1 April over 120 days each year. The first samples were taken after 60 days, while the second occasion of sampling was at the end of the experiment period. The plants were cut on each occasion from half of the area of the culture pot (2 dm^2). In the material of the samples the living and dead plant parts were separated; the living material was sorted into fractions of dominant or prevalent species and of other species, respectively. The living parts represent the net aboveground phytobiomass production, while the living and dead plant parts together the net phytomass production; both are given in dry weight (PRÉCSÉNYI 1975). For the determination of the amount of drymatter the material of the samples was dried at 80°C to body-balance.

For lack of the necessary equipment there was no possibility to determine the energy content of the plant parts for the estimation of the energy utilization or ecological efficiency, therefore data of the relevant literature were taken into consideration (GOLLEY 1961, LIETH

Table 1
Quantitative data on aboveground drymatter production
 (g · dm⁻²)

| Composition | Light treatments | | | | | |
|---|------------------|--------|-----------|--------|----------------|--------|
| | blue light | | red light | | compound light | |
| | 1 | 2 | 1 | 2 | 1 | 2 |
| <i>S.-F. r. p. festucetosum rupicolae</i> | | | | | | |
| Living plant parts: | | | | | | |
| <i>Festuca rupicola</i> | 0.3677 | 1.2132 | 0.4357 | 2.1668 | 0.4585 | 2.0945 |
| Other species | 0.2594 | 0.7087 | 0.7514 | 1.5498 | 0.1992 | 0.8947 |
| Necrotized plant parts | 0.0200 | 0.7300 | 0.0000 | 1.2200 | 0.1150 | 0.4100 |
| Total | 0.6471 | 2.6519 | 1.1871 | 4.9366 | 0.7727 | 3.3992 |
| <i>S.-F. r. p. stipetosum capillatae</i> | | | | | | |
| Living plant parts: | | | | | | |
| <i>Stipa capillata</i> | 0.5031 | 0.2365 | 1.2306 | 2.6288 | 0.5684 | 1.7640 |
| <i>Salvia nemorosa</i> | 0.1918 | 0.3868 | 0.4318 | 1.7540 | 0.1747 | 0.4936 |
| Other species | 0.1268 | 0.4677 | 0.3621 | 0.6352 | 0.3508 | 0.4695 |
| Necrotized plant parts | 0.0000 | 0.4900 | 0.1030 | 1.4600 | 0.1025 | 0.6250 |
| Total | 0.8217 | 1.5810 | 2.1275 | 6.4780 | 1.1964 | 3.3521 |
| <i>S.-F. r. p. andropogonetosum</i> | | | | | | |
| Living plant parts: | | | | | | |
| <i>Bothriochloa ischaemum</i> | 0.3773 | 0.2315 | 1.6950 | 2.9812 | 0.5040 | 0.8286 |
| Other species | 0.2604 | 0.9085 | 0.4461 | 1.0702 | 0.2190 | 0.7996 |
| Necrotized plant parts | 0.1050 | 1.2015 | 0.0625 | 3.8300 | 0.0950 | 2.2500 |
| Total | 0.7427 | 2.3415 | 2.2036 | 7.8814 | 0.8180 | 3.8782 |
| <i>S.-F. r. p. poëtosum angustifoliae</i> | | | | | | |
| Living plant parts: | | | | | | |
| <i>Poa angustifolia</i> | 0.5835 | 1.0984 | 0.6609 | 3.0268 | 0.5193 | 1.9388 |
| <i>Achillea collina</i> | 0.2073 | 0.0616 | 0.6875 | 0.6048 | 0.3799 | 0.0774 |
| Other species | 0.1952 | 1.0720 | 0.6461 | 2.0952 | 0.7077 | 0.7672 |
| Necrotized plant parts | 0.0265 | 0.5800 | 0.0000 | 1.7900 | 0.0550 | 1.0400 |
| Total | 1.0125 | 2.8120 | 1.9945 | 7.5168 | 1.6619 | 3.8234 |
| Average values of drymatter production | -0.8060 | 2.3466 | 1.8782 | 6.7032 | 1.1123 | 3.6132 |

Note: 1 = after 60 days; 2 = after 120 days.

1968, MOIR 1969, PRÉCSÉNYI 1975, etc.). On this basis 1 g drymatter of plant was regarded as equivalent to 17580 J energy.

The chlorophyll concentration was determined in those species of each subassociation which had the largest share from the aboveground phytobiomass. They were (Soó 1968, 1970, 1973, 1980, Soó and KÁRPÁTI 1968):

- *Salvio-Festucetum rupicolae pannonicum festucetosum rupicolae*: *Festuca rupicola* Heuff.;
- *S.-F. r. p. stipetosum capillatae*: *Stipa capillata* L. and *Salvia nemorosa* L.;
- *S.-F. r. p. andropogonetosum*: *Bothriochloa ischaemum* (L.) Keng.;
- *S.-F. r. p. poëtosum capillatae*: *Poa angustifolia* L. and *Achillea collina* Becker in Rehb.

The photosynthetic pigments were extracted with acetone—with several replications—from the fresh plant material at the time of sampling, then washed over into petroleum ether.

The absorption of the pigment extract was measured by a Spekol spectrophotometer at 647 and 664 nm wave-lengths, and the amount of chlorophyll (chlorophyll *a* + *b*) determined after ZIEGLER and EGLE (1965). In the course of processing the data the average values of repeated measurements were taken into account. For the sake of a more reliable comparison—knowing the drymatter concentrations—we converted the measuring data obtained for unit weight of fresh material to dry weight figures too.

The variance analysis and the correlation examinations employed in evaluating the experiment results were based on the work of SVÁB (1973).

In the paper we reckon with the mean values of the results of the three experiment series, since no significant differences were found between the data obtained in the different years.

Results

Aboveground drymatter production, and energy utilization

The quantitative data of aboveground phytomass production, separated to fractions, at the time of sampling are summarized by stand and light treatment in Table 1 and represented in Fig. 1.

The largest amount of drymatter was obtained with plant stands raised in red light (the average weight of phytomass was $1.8782 \text{ g} \cdot \text{dm}^{-2}$ on the first and $6.7032 \text{ g} \cdot \text{dm}^{-2}$ on the second occasion of sampling), the smallest one with those grown in blue light (mean values: $0.8060 \text{ g} \cdot \text{dm}^{-2}$ and $2.3466 \text{ g} \cdot \text{dm}^{-2}$, respectively). The amount of drymatter produced in compound "white" light was closer to that obtained in blue light ($1.1123 \text{ g} \cdot \text{dm}^{-2}$ and $3.6132 \text{ g} \cdot \text{dm}^{-2}$, respectively).

The share of the phytobiomass of each species from the total aboveground phytobiomass is shown—according to the different light treatments—in Fig. 2.

Out of the species occurring in the highest proportion *Festuca rupicola* and *Stipa capillata* had the largest share in the drymatter of living plant parts when grown in compound "white" light, *Salvia nemorosa* and *Poa angustifolia* when raised in blue light, and *Achillea collina* and *Botriochloa ischaemum* when treated with red light.

For net productivity on the basis of aboveground phytomass, and energy utilization relative to the total radiation (efficiency) detailed data are given in Table 2. Considering the full period of the experiment, the highest average productivity was obtained in red light (daily value: $+0.0559 \text{ g} \cdot \text{dm}^{-2}$), the lowest as an effect of blue light (daily average $+0.0196 \text{ g} \cdot \text{dm}^{-2}$), while in the case of compound "white" light the daily mean value of productivity was $+0.0310 \text{ g} \cdot \text{dm}^{-2}$. The proportions of energy utilization are similar. The values of efficiency: 3.72 per cent in red light, 1.30 per cent in blue light, and 2.01 per cent as the effect of compound "white" light used.

Chlorophyll contents in the dominant species

The chlorophyll *a* + *b* concentrations per unit weight of the fresh plant material in the six species examined are given in Fig. 3. In general, the differences were not great between the light treatments, the mean values were nearly identical: $3.240 \mu\text{g} \cdot \text{mg}^{-1}$ with blue, $3.268 \mu\text{g} \cdot \text{mg}^{-1}$ with red and $3.993 \mu\text{g} \cdot \text{mg}^{-1}$ with compound "white" light after 60 days; $3.672 \mu\text{g} \cdot \text{mg}^{-1}$ with blue, $3.129 \mu\text{g} \cdot \text{mg}^{-1}$ with red and $3.480 \mu\text{g} \cdot \text{mg}^{-1}$ with compound "white" light at the end of the experiment.

Chlorophyll concentrations calculated for dry weight are contained in Table 3; the lowest, highest and average values obtained with the light treatments are shown in Fig. 4.

As for the mean values of the chlorophyll contents of species on dry weight basis, differences between the light treatments are not considerable either. At the end of the experi-

ment the chlorophyll concentrations were almost in every case lower than those determined after 60 days. The decrease was the greatest in the case of the compound "white" light, while in plant species raised in blue and red light it was negligible.

The chlorophyll contents of the phytobiomass of species having a decisive or relatively large share in the aboveground drymatter production, as calculated from concentrations characteristic of the dry weight are summed up in Table 4. In Fig. 5 the amounts of phytobiomass and chlorophyll per unit area are shown.

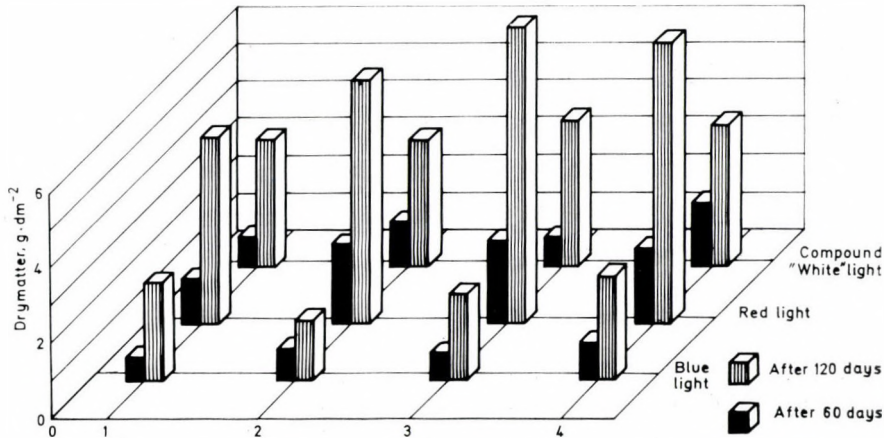


Fig. 1. Aboveground drymatter production. 1. *Salvio-Festucetum rupicolae pannonicum festucetosum rupicolae*, 2. *S.-F. r. p. stipetosum capillatae*, 3. *S.-F. r. p. andropogonetosum*, 4. *S.-F. r. p. poëtosum angustifoliae*

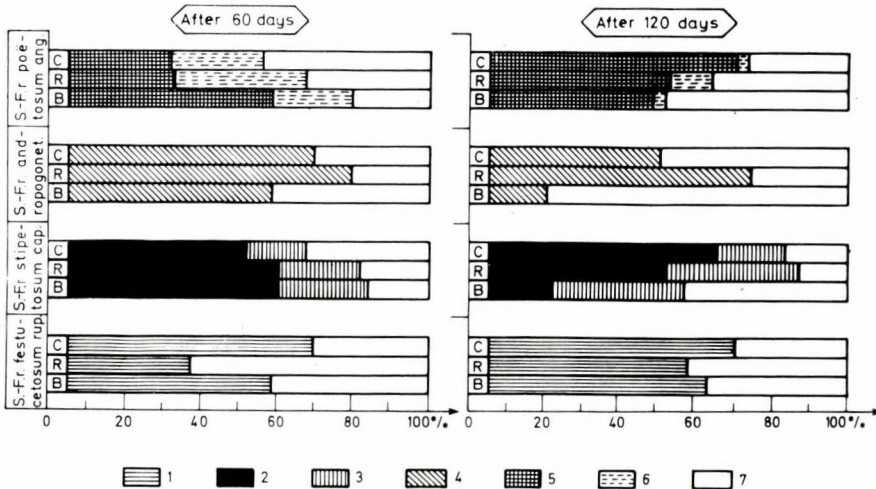


Fig. 2. Share of the phytobiomass of species in the aboveground phytobiomass production. 1. *Festuca rupicola*, 2. *Stipa capillata*, 3. *Salvia nemorosa*, 4. *Bothriochloa ischaemum*, 5. *Poa angustifolia*, 6. *Achillea collina*, 7. Phytobiomass of other species; B: blue light, R: red light, C: compound "white" light

Table 2

Aboveground net productivity ($\text{g} \cdot \text{dm}^{-2}$ per day) and energy

| Examination intervals | <i>S.-F. r. p. festucetosum rupicola</i> | | <i>S.-F. r. p. stipetosum capillata</i> | |
|-------------------------|--|------|---|------|
| | product. | eff. | product. | eff. |
| In response | | | | |
| In 60 days | +0.0113 | 0.72 | +0.0137 | 0.91 |
| Between 60 and 120 days | +0.0334 | 2.23 | +0.0127 | 0.84 |
| In 120 days | +0.0221 | 1.47 | +0.0132 | 0.88 |
| In response | | | | |
| In 60 days | +0.0198 | 1.32 | +0.0355 | 2.36 |
| Between 60 and 120 days | +0.0625 | 4.17 | +0.0725 | 4.83 |
| In 120 days | +0.0411 | 2.74 | +0.0540 | 3.60 |
| In response to | | | | |
| In 60 days | +0.0129 | 0.86 | +0.0199 | 1.33 |
| Between 60 and 120 days | +0.0438 | 2.92 | +0.0359 | 2.39 |
| In 120 days | +0.0283 | 1.89 | +0.0279 | 1.86 |

Table 3

Chlorophyll concentrations on dry weight basis
($\mu\text{g} \cdot \text{mg}^{-1}$)

| Species | After 60 days' light treatment | | | After 120 days' light treatment | | |
|-------------------------------|-----------------------------------|--------|----------|------------------------------------|--------|----------|
| | blue | red | compound | blue | red | compound |
| <i>Salvia nemorosa</i> | 21.365 | 21.442 | 25.301 | 16.433 | 17.704 | 11.377 |
| <i>Achillea collina</i> | 16.505 | 17.255 | 21.234 | 14.945 | 13.763 | 15.372 |
| <i>Festuca rupicola</i> | 11.873 | 7.712 | 13.601 | 10.186 | 9.064 | 9.521 |
| <i>Poa angustifolia</i> | 11.929 | 8.730 | 10.556 | 8.957 | 8.530 | 8.822 |
| <i>Stipa capillata</i> | 15.918 | 9.781 | 13.409 | 14.912 | 7.370 | 10.961 |
| <i>Bothriochloa ischaemum</i> | 14.967 | 12.644 | 11.456 | 19.561 | 9.730 | 9.298 |
| Average values | 15.426 | 12.927 | 15.926 | 14.166 | 11.027 | 10.892 |

utilization relative to total radiation (efficiency, in %)

| <i>S.-F. r. p. andropogonetosum</i> | | <i>S.-F. r. p. poëtosum angustifoliae</i> | | Mean values | |
|---|------|---|------|-------------|------|
| product. | eff. | product. | eff. | product. | eff. |
| to blue light | | | | | |
| +0.0124 | 0.83 | +0.0169 | 1.12 | +0.0134 | 0.90 |
| +0.0266 | 1.78 | +0.0030 | 2.00 | +0.0257 | 1.71 |
| +0.0195 | 1.30 | +0.0234 | 1.56 | +0.0196 | 1.30 |
| to red light | | | | | |
| +0.0367 | 2.45 | +0.0332 | 2.22 | +0.0313 | 2.09 |
| +0.0946 | 6.31 | +0.0920 | 6.13 | +0.0804 | 5.36 |
| +0.0657 | 4.38 | +0.0626 | 4.17 | +0.0559 | 3.72 |
| compound "white" light | | | | | |
| +0.0136 | 0.91 | +0.0277 | 1.85 | +0.0185 | 1.24 |
| +0.0510 | 3.40 | +0.0360 | 2.40 | +0.0417 | 2.78 |
| +0.0323 | 2.15 | +0.0319 | 2.12 | +0.0310 | 2.01 |

Table 4

Chlorophyll content of aboveground phytobiomass in the dominant species
(mg · dm⁻²)

| Plant stands — species | Light treatments — sampling times | | | | | |
|---|-----------------------------------|-------------------|------------------|-------------------|------------------|-------------------|
| | blue light | | red light | | compound light | |
| | after 60 days | after 120 days | after 60 days | after 120 days | after 60 days | after 120 days |
| <i>S.-F. r. p. festucetosum rupicolae</i> | | | | | | |
| — <i>Festuca rupicola</i> | 4.36 | 12.36 | 3.36 | 19.64 | 6.24 | 19.94 |
| <i>S.-F. r. p. stipetosum capillatae</i> | | | | | | |
| — <i>Stipa capillata</i> | 8.01 | 3.53 | 12.04 | 19.37 | 7.62 | 19.33 |
| — <i>Salvia nemorosa</i> | 4.10 | 6.36 | 9.26 | 31.05 | 4.42 | 5.61 |
| <i>S.-F. r. p. andropogonetosum</i> | | | | | | |
| — <i>Bothriochloa ischaemum</i> | 5.65 | 4.53 | 21.43 | 29.01 | 5.77 | 7.70 |
| <i>S.-F. r. p. poëtosum angustifoliae</i> | | | | | | |
| — <i>Poa angustifolia</i> | 6.96 | 9.84 | 5.77 | 25.82 | 5.48 | 17.10 |
| — <i>Achillea collina</i> | 3.42 | 0.92 | 11.86 | 8.32 | 8.07 | 1.19 |
| Average values: | 5.42 | 6.26 | 10.62 | 22.20 | 6.27 | 11.81 |

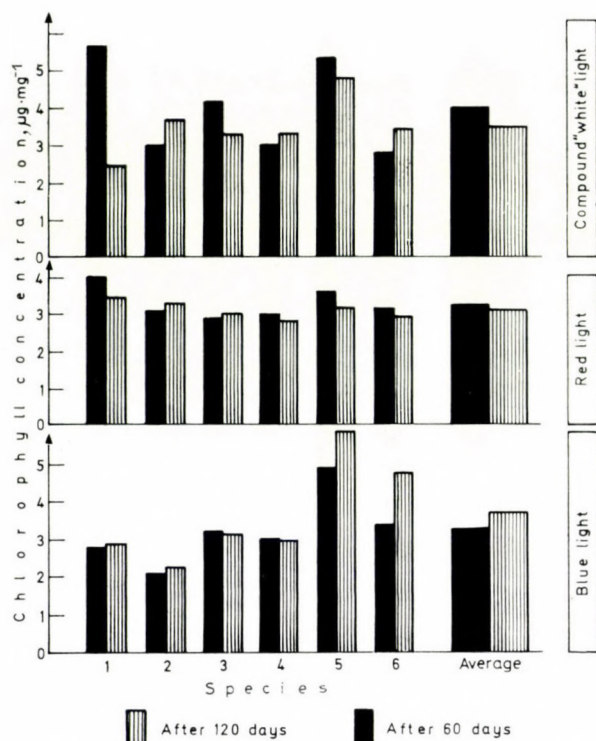


Fig. 3. Chlorophyll concentrations characteristic of the dominant species in the fresh plant material. 1. *Salvia nemorosa*, 2. *Achillea collina*, 3. *Festuca rupicola*, 4. *Poa angustifolia*, 5. *Stipa capillata*, 6. *Bothriochloa ischaemum*

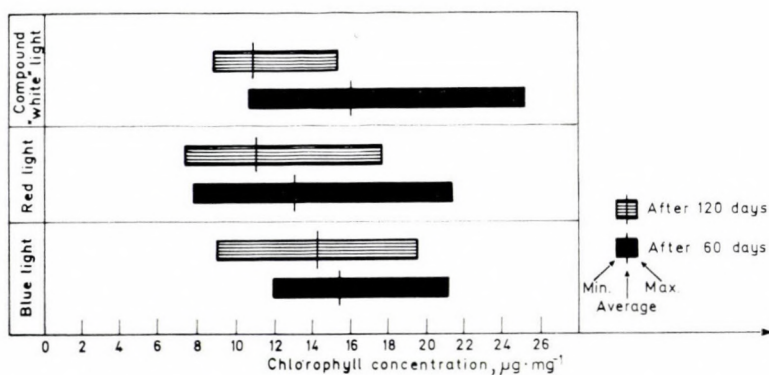


Fig. 4. Lowest, highest and average chlorophyll concentrations of the six dominant plant species on dry weight basis

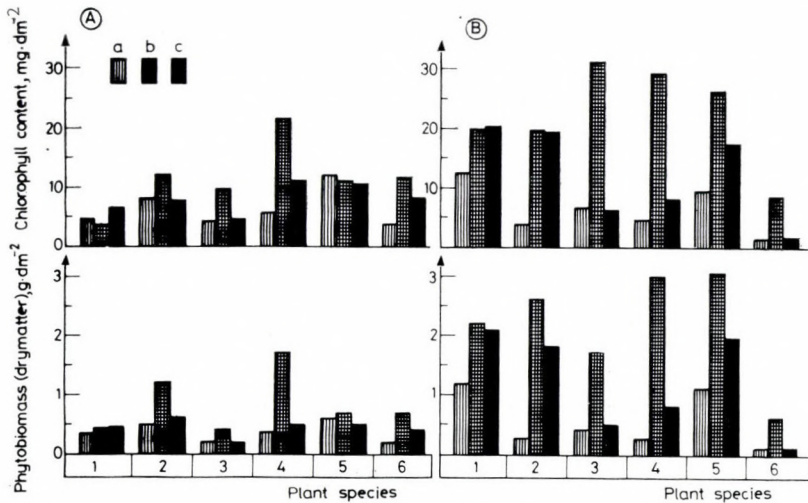


Fig. 5. Aboveground phytobiomass production in the dominant species, and chlorophyll content of the phytobiomass. 1. *Festuca rupicola*, 2. *Stipa capillata*, 3. *Salvia nemorosa*, 4. *Bothriochloa ischaemum*, 5. *Poa angustifolia*, 6. *Achillea collina*; a: blue light, b: red light, c: compound "white" light; A: after 60 days, B: after 120 days

Discussion

The amounts of drymatter produced in blue, red and compound "white" light were compared by variance analysis. The results are given in Table 5.

Under the influence of lights of different spectral composition significant differences were found—after 60 and 120 days alike—to occur both in the amount of the phytomass and between the phytobiomass quantities of the dominant species. The greatest difference in average net production was pointed out between plant stands grown in blue and red light, as well as between those raised in compound "white" and red light, especially at the end of the experiment. The amounts of phytomass produced in compound "white" and blue light, respectively, showed essential differences only on the second occasion of sampling, and even then at a low (10 per cent) level of probability. Between the dominant species no significant differences in the amount of phytobiomass were found here. In blue light smaller amounts of phytomass and phytobiomass were produced than in compound light.

The trend of phytomass production observed in our experiment as a response to various spectral compositions of light can be compared to other authors' results only with restrictions. The comparison is made difficult by the mostly essential differences in the conditions of the experiments, first of all in the period of examination and the energy content of light, but difficulties

Table 5
Comparison of drymatter production by variance analysis

| Light effects compared | | Average difference (g · dm ⁻²) | | Significant difference (SD) | | Probability level | |
|------------------------|------------|---|---|--------------------------------|---|----------------------|--|
| 1 | 2 | in phytomass | in the phytobio- mass of dominant species | in phytomass | in the phytobio- mass of dominant species | in phyto- mass | in the phyto- biomass of dom- inant species |
| after 60 days | | | | | | | |
| Compound light | blue light | -0.3062 | -0.0623 | — | — | — | — |
| Compound light | red light | +0.7660 | +0.4228 | +0.5936 | +0.3874 | 5% | 5% |
| Blue light | red light | +1.0722 | +0.4851 | +0.8537 | +0.3874 | 1% | 5% |
| after 120 days | | | | | | | |
| Compound light | blue light | -1.2666 | -0.6615 | -1.0866 | — | 10% | — |
| Compound light | red light | +3.0900 | +0.9942 | +2.8382 | +0.7821 | 0.1% | 10% |
| Blue light | red light | +4.3566 | +1.6557 | +2.8382 | +1.3183 | 0.1% | 1% |

Note: The amount produced by the treatment in column 2 in comparison to that in column 1: + = larger, — = smaller.

are also caused by the fact that the drymatter data are not generally given in terms of unit area. In spite of this the phytomass production obtained in the different light treatments in our experiment followed much the same order as established by the authors listed in the introduction. For example, HOOVER (1937), GABRIELSEN (1948), VINCE and STOUGHTON (1957) and KLESCHNIN (1960) found that with a radiation of the same energy the intensity of photosynthesis in the leaves of many dicotyledonous and monocotyledonous plants was substantially greater in the orange and red wave ranges than in the blue-violet one. KWACK and DUNN (1961) observed with *Pisum sativum*, STEVENSON and DUNN (1965) with *Lycopersicon esculentum* and SZÁSZ, HORVÁTH and SZ. BARSÍ (1968) with *Glycine soja* that at the same energy level and light intensity, respectively, a larger amount of drymatter was produced in red than in blue light. In the experiments of WARRINGTON and MITCHELL (1976) — including the species of *Trifolium*, *Glycine*, *Sorgum* and *Lolium* — an increase in the red wave range of the compound “white” light resulted in a similar increase in the dry weight of plants, while with the growing proportion of the blue colour the amount of drymatter decreased.

As to such results as differing from our findings (e.g. VOSKRESENSKAYA and GRISHINA 1959, KLESCHNIN 1960, HORVÁTH and V. FEHÉR 1963, KOLTAY and HORVÁTH 1967) it is worth being mentioned that the spectral composition of light acts on the primary production in close correlation with the energy

level. At different energy levels the spectral effect may be highly varied — often even the opposite; and it is also important that the drymatter production in the different species is not uniformly influenced by the spectral composition of light (HORVÁTH 1965, KOLTAY and HORVÁTH 1967). The latter has been proved by the results of our experiment too. The species composing the plant stands gave differentiated responses to the spectral composition, as clearly seen from the fact that their share in the phytobiomass production generally varied with the light treatment, and — with some of them (with *Poa angustifolia* and *Stipa capillata* in particular) — even depended on the age of the plants.

We found that the energy of the red light was much better utilized than that of the blue light. It can probably be explained by the fact that at the same energy level — a condition in our experiment too — the number of photons of the red light is about one and a half times higher than that of the blue light, and the *in vivo* light absorption of the leaves of plants is also greater in the red spectral range than in the blue one (NUERNBERGK 1961), so that the chlorophyll molecules can absorb an essentially larger number of photons in red light. Namely, the amount of net drymatter produced depends first of all on the number of photons fixed by the plants, as proved e.g. by the experiments of HORVÁTH et al. (1973), who obtained the same extent of drymatter accumulation in *Sinapis alba* plants when the energy of the blue light was 1.79 times that of the red light. This kind of light treatment ensured that the *in vivo* light absorption of the leaves was almost uniform concerning the number of photons. The light energy fixed was then higher in blue than in red light, but the energy utilization was essentially lower.

The six plant species that had the greatest share in the phytobiomass production showed no significant differences in response to various light treatments, in respect of either the average values of chlorophyll content in the fresh plant material, or the average chlorophyll concentrations on dry weight basis. This was most conspicuous in the red light treatment in which the largest amount of drymatter was produced; at the same time the average chlorophyll concentration in the fresh plant material was the lowest under the influence of red light: in the first half of the experiment its value on dry weight basis was lower than in the other treatments, and even at the end hardly exceeded the minimum value measured with compound light. This again suggests that the productivity of the vegetation greatly depends on the number of photons absorbed by the chlorophyll molecules.

We tried to find correlation between the phytobiomass productions per unit area in the dominant species and their chlorophyll contents with regression analysis. The results are shown in Fig. 6. Accordingly there is a very close linear regression correlation between the phytobiomass quantities and the chlorophyll contents. The values of the correlation coefficient are: $r =$

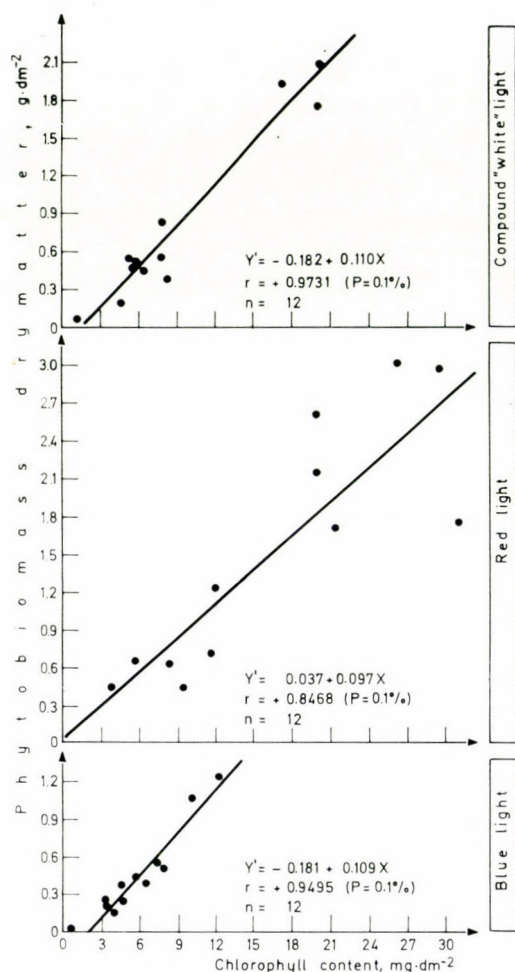


Fig. 6. Relationship between the aboveground phytobiomass production and the chlorophyll content of the phytobiomass in the dominant species

$= +0.9731$ with the compound "white", $r = +0.9495$ with the blue and $r = +0.8468$ with the red light. The probability level of significance is in each case 0.1%. A similar close positive correlation was pointed out between the drymatter production and the chlorophyll content e.g. by BROUGHAM (1960), WHITTAKER and GARFINE (1962), MEDINA and LIETH (1963, 1964), OVINGTON and LAWRENCE (1967) and ENDRÉDI and HORVÁTH (1977) and between the amount of drymatter and that of chlorophyll-*a* by RIGAU and BERBEL (1972) and RIGAU (1977).

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CHARACTERISTICS OF PHOTOSYNTHETIC INTENSITY AND EFFICIENCY IN SPECIES OF A TURKEY OAK-OAK PHYTOCENOSIS*

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The paper discusses the photosynthetic intensity and efficiency of 12 plant species in the "Síkfőkút Project" Turkey oak-oak phytocenosis. The results are based on the photosynthetic fixation of $^{14}\text{CO}_2$. The lowest intensity of photosynthesis was characteristic of *Quercus petraea*, the highest one—maximum $3.6 \text{ mg dm}^{-2} \text{ h}^{-1}$ —of *Ligustrum vulgare*. Besides *Ligustrum vulgare*, *Crataegus oxyacantha*, *Lonicera xylosteum*, *Euonymus verrucosus* and *Acer tataricum* showed heliophilous character in photosynthesis. The photosynthetic activity of *Viburnum lantana* and *Bromus ramosus* was more intensive in poorer light. At low temperatures ($7-7.5^\circ\text{C}$) in autumn the photosynthesis of all species, except *Bromus ramosus*, was sharply reduced. The percentage distribution of photosynthetic intensity values by parts of the day, season as well as according to the rate of illumination is shown in tables. The efficiency of photosynthesis was highest in summer. The efficiency values generally increased from morning to evening.

The space lattice diagram shows the relation of photosynthetic intensity to light energy and temperature. Finally, some data calculated on the basis of the results of CO_2 fixation refer to longer periods of photosynthesis.

Introduction

Our work is connected with the forest ecosystem research carried on in Hungary on the "Síkfőkút Project".

The subject is: "Complex investigations of an oak forest ecosystem from the natural to the culture state on a hilly model area".

The subject pertains to the main research line "Protection of man and biosphere (MAB)". The main task of this climatic zonal oak forest research is to size up the organic matter production and explore the material- and energy turnover of the phytocenosis in relation to the effects of ecofactors.

Our objective was to establish the photosynthetic characteristics of 12 plant species representing a considerable proportion of the phytocenosis: the intensity and efficiency of their photosynthetic activities in different periods and under different environmental conditions.

Characterization of the model area, material and method

The growing stock of the Turkey oak-oak forest association (*Quercetum petraeae-cerris*) of the model area is an about 70 years old second growth. Of the two denominative species *Quercus petraea* occurs in 84, *Quercus cerris* in 16 per cent. The number of trunks is 816/ha, the canopy is closed to 75-80 per cent. The average height of trees is 17-18 m. The shrub

* "Síkfőkút Project" No. 89.

Table 1
*Characteristic and dominant species
 of the Turkey oak-oak forest ecosystem*

| Test plants | | |
|---------------|-----------------------------|-------------------------------|
| Serial number | Specific name | Abbreviation of specific name |
| 1 | <i>Quercus petraea</i> | Q. p. |
| 2 | <i>Quercus cerris</i> | Q. c. |
| 3 | <i>Ligustrum vulgare</i> | L. v. |
| 4 | <i>Cornus mas</i> | C. m. |
| 5 | <i>Acer campestre</i> | A. c. |
| 6 | <i>Euonymus europaeus</i> | E. e. |
| 7 | <i>Euonymus verrucosus</i> | E. v. |
| 8 | <i>Acer tataricum</i> | A. t. |
| 9 | <i>Viburnum lantana</i> | V. l. |
| 10 | <i>Lonicera xylosteum</i> | L. x. |
| 11 | <i>Crataegus oxyacantha</i> | C. o. |
| 12 | <i>Bromus ramosus</i> | B. r. |

stratum is rich, the number of stems per hectare is over 93 thousand (JAKUCS et al. 1973, JAKUCS 1978). On the model area forest cultivation has not been carried on for 20 years.

For test plants we chose species dominant in quantity and characteristic of the Turkey oak-oak forest association (Table 1).

From fully developed leaves of the test plants average samples were taken, some 30–40 leaves per species. For the purpose of measuring the intensity of photosynthesis 30 discs of 7 mm Ø were cut out of them by means of a corkscrew. The discs were placed on wet filter paper and put in the photosynthesis apparatus where the photosynthetic fixation of $^{14}\text{CO}_2$ was carried out. The intensity of photosynthesis was determined by measuring the radioactivity of the leaf discs. From the values of radioactivity the quantity of CO_2 uptake was converted into mg. For the description of the apparatus, procedure and calculation see: SUBA J. et al. (1982). The time- and microclimatic data of CO_2 fixation are contained in Table 2. The CO_2 concentration was increased fourfold compared to the average and could thus be considered a stable factor favourable for the photosynthetic process. The water supply was optimum.

The photosynthetic intensity of the species examined was measured under the light conditions of the herb- and tree stratum (at a height of 2 and 20 m, respectively, from the ground level).

The results are regarded as semiquantitative, giving a sound basis for comparison. The values obtained are close to the net photosynthesis values. It must be taken into consideration that during the one-hour period of fixation a minor extent of reassimilation of CO_2 contained in the gas mixture may also occur. VOZNESENSKI (1971) suggested to distinguish the carbon-14 method from other techniques, e.g. from the "infrared" gas analysis, even as regards its results.

In calculating the value of efficiency (E) expressing the photosynthetic utilization of light energy we used the following formula:

$$E_{\%}^{\text{O}} = \frac{\text{fixed CO}_2 (\text{nmol} \cdot \text{dm}^{-2} \cdot \text{h}^{-1}) \times \text{energy required for the incorporation of 1 nmol CO}_2}{\text{incident light energy (Joule dm}^{-2} \cdot \text{h}^{-1})} \times 100$$

Table 2
Time and microclimatic data of CO₂ fixation

| | 8-9 a.m. | | | 1-2 p.m. | | | 6-7 p.m. | | |
|----------------------------------|----------|--------|---|----------|--------|---|----------|------|---|
| | °C | Lux | Joule dm ⁻² · h ⁻¹ | °C | Lux | Joule dm ⁻² · h ⁻¹ | °C | Lux | Joule dm ⁻² · h ⁻¹ |
| <i>Spring (1981)**</i> | | | | | | | | | |
| 17 June in shade (2 m*) | 17.9 | 342 | 753 | 20.9 | 544 | 1 004 | 19.3 | 151 | 335 |
| 18 June in sun- shine (20 m*) | 20.7 | 45 800 | 21 181 | 19.8 | 33 140 | 11 720 | 18.9 | 9170 | 6697 |
| <i>Summer (1980)**</i> | | | | | | | | | |
| 19 June in shade (2 m*) | 19.9 | 550 | 1 172 | 23.9 | 1 220 | 1 674 | 18.8 | 11.5 | 84 |
| 20 July in sun- shine (20 m*) | 23.2 | 31 330 | 20 030 | 23.7 | 22 830 | 5 776 | 21.7 | 438 | 209 |
| <i>Autumn (1980)</i> | | | | | | | | | |
| 3 October in shade | 7.4 | 975 | 2 051 | 9.8 | 1 200 | 1 004 | 6.2 | 7.4 | 42 |
| 4 October in sun-shine | 7.7 | 41 800 | 21 558 | 10.5 | 10 625 | 10 381 | 9.2 | 3193 | 963 |

*The photosynthetic intensity was measured under field conditions in natural light. 2 m and 20 m mean the heights above ground level where the CO₂ fixation took place.

** By summer time.

The energy required for the incorporation of 1 nmol CO₂ was taken for 0.0047 Joule. According to BOLTON (1978) 52 per cent of the total incident light energy measured in Joule falls within the range of photosynthetically active radiation (PHAR). The efficiency values given in the paper are based on the PHAR.

Results and evaluation

The photosynthetic intensity of the species was summed up on the basis of the results of measuring at 18 different times (Fig. 1). Very low photosynthetic activity was characteristic of *Quercus petraea* (Fig. 2) which incorporated an average of 0.08 mg CO₂ dm⁻² · h⁻¹. The highest photosynthetic performance was shown by *Ligustrum vulgare* (Fig. 3) with a 0.64 mg CO₂ dm⁻² · h⁻¹ uptake, 7.8 times as much as the former. The rest of the species can be placed in three categories:

The intensity of photosynthesis is:

| low | medium | high |
|---------------------------|-----------------------|---|
| 0.15-0.28 | 0.35-0.37 | 0.45-0.51 mg CO ₂ dm ⁻² · h ⁻¹ |
| <i>Bromus ramosus</i> | <i>Acer tataricum</i> | <i>Euonymus verrucosus</i> |
| <i>Quercus cerris</i> | <i>Acer campestre</i> | <i>Lonicera xylosteum</i> |
| <i>Euonymus europaeus</i> | <i>Cornus mas</i> | <i>Crataegus oxyacantha</i> |
| <i>Viburnum lantana</i> | | |

The correlations of the photosynthetic characteristics of the species are indicated by the diagrams of the cluster analysis (Fig. 4). The analyses were made with the Odra 1204 type computer of the Ho-Shi-Minh Teachers Training College, Eger, according to the ALGOL programme.

The correlation is the closest between *Ligustrum vulgare* and *Euonymus europaeus* (Fig. 4a). Their photosynthetic intensities change at a similar rate, though with *Ligustrum* in a higher order of magnitude. The correlation is close — 90 per cent — between *Euonymus verrucosus* and *Crataegus oxyacantha*; here the absolute values of photosynthetic intensity are also nearly equal. The lowest correlation is shown by *Bromus ramosus* to which *Quercus petraea* is the closest, with 57 per cent. As for the related species, the correlation between *Quercus petraea* and *Q. cerris* is 76 per cent.

The cluster diagram in Fig. 4b shows the correlations of photosynthetic intensity by order, on the basis of Euclidean distance function.

The following species are closest to one another:

Euonymus europaeus — *Viburnum lantana*
Quercus petraea — *Bromus ramosus*
Crataegus oxyacantha — *Euonymus verrucosus*
Crataegus oxyacantha — *Lonicera xylosteum*
Crataegus oxyacantha — *Cornus mas*

Owing to its outstanding photosynthetic productivity *Ligustrum vulgare* is farthest from the other species.

The seasonal distribution of the photosynthetic intensity is seen in Table 3.

On the basis of the available light energy (Joule) it can be established that the efficiency of photosynthesis is much higher in summer than in spring. *Ligustrum vulgare*, in particular, was found to increase its photosynthetic activity to a considerable extent, almost twofold compared to the spring value; greatly increased the performances of *Euonymus verrucosus* and *Crataegus oxyacantha* too. *Bromus ramosus* fixed the largest amount of CO₂ in autumn (Fig. 5). The difference in the value of photosynthesis between herb- and tree stratum is the greatest, some 10-fold in spring, decreasing to 5.3 in summer, and is 7.6-fold in autumn.

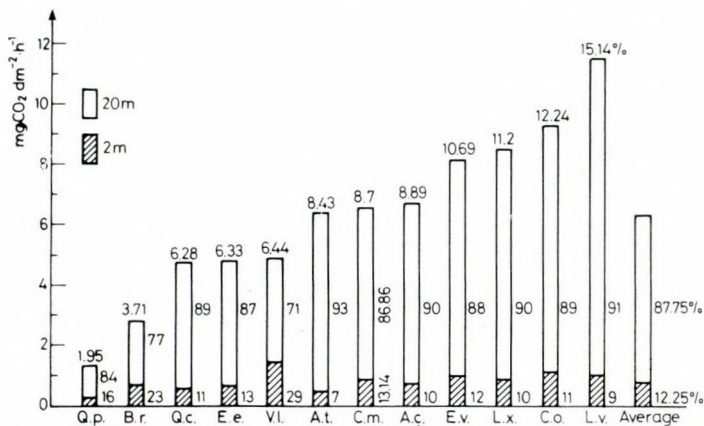


Fig. 1. Absolute values of photosynthetic intensity and their percentage distribution among the species. The numbers beside the columns refer to the ratios of values obtained under the light conditions of the tree- and herb stratum

Table 3

Seasonal distribution of photosynthetic intensity
on the basis of all data of measuring

| Season | Herb stratum | Tree stratum | Total | Total incident energy (Joule) |
|--------|-----------------|-----------------|-------|--|
| | | | | |
| Spring | 3.54 | 39.09 | 42.6 | 38.7 |
| Summer | 7.03 | 36.2 | 43.3 | 27.8 |
| Autumn | 1.23 | 12.66 | 14.1 | 33.5 |
| Total | 11.8 | 87.95 | 100.0 | 100.0 |

Acer tataricum fixed very little CO_2 with the poor light conditions of the herb stratum, and so the differences between the two strata were greater. This is related with the heliophilous nature of the species. The photosynthesis of *Viburnum lantana* in the shaded herb stratum was characterized in every case by a much higher production compared to the other species.

Quercus petraea

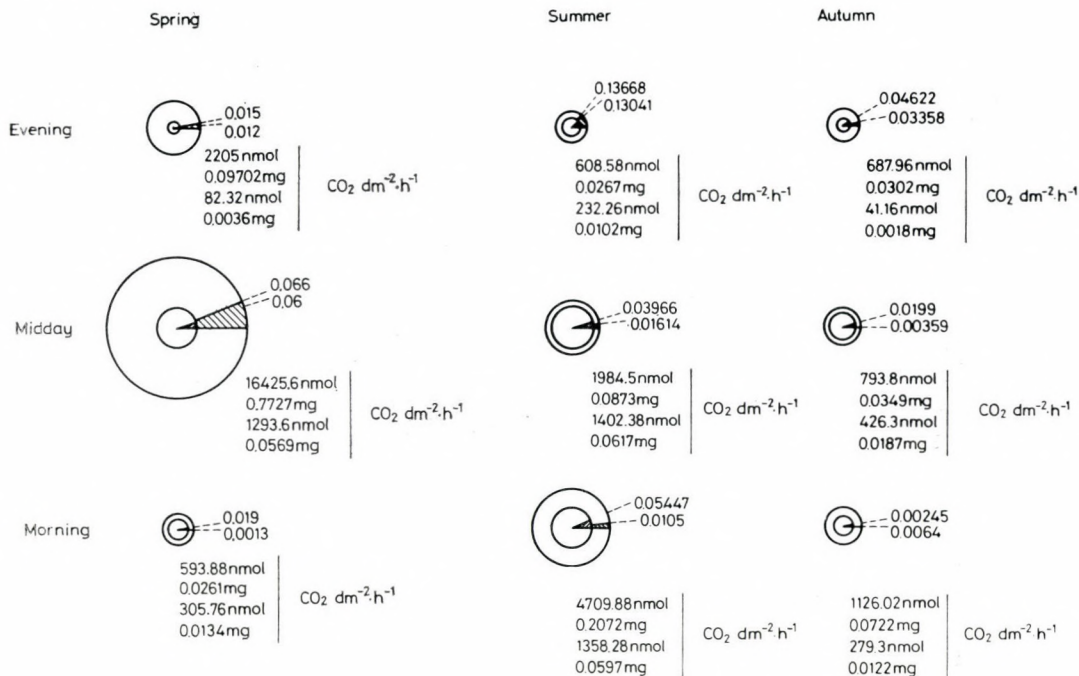


Fig. 2. CO_2 fixation values in *Quercus petraea*. The outer circle represents the photosynthetic results for the tree- (20 m), the inner circle for the herb stratum (2 m), in proportion to area. The striped sectors refer to the efficiency values

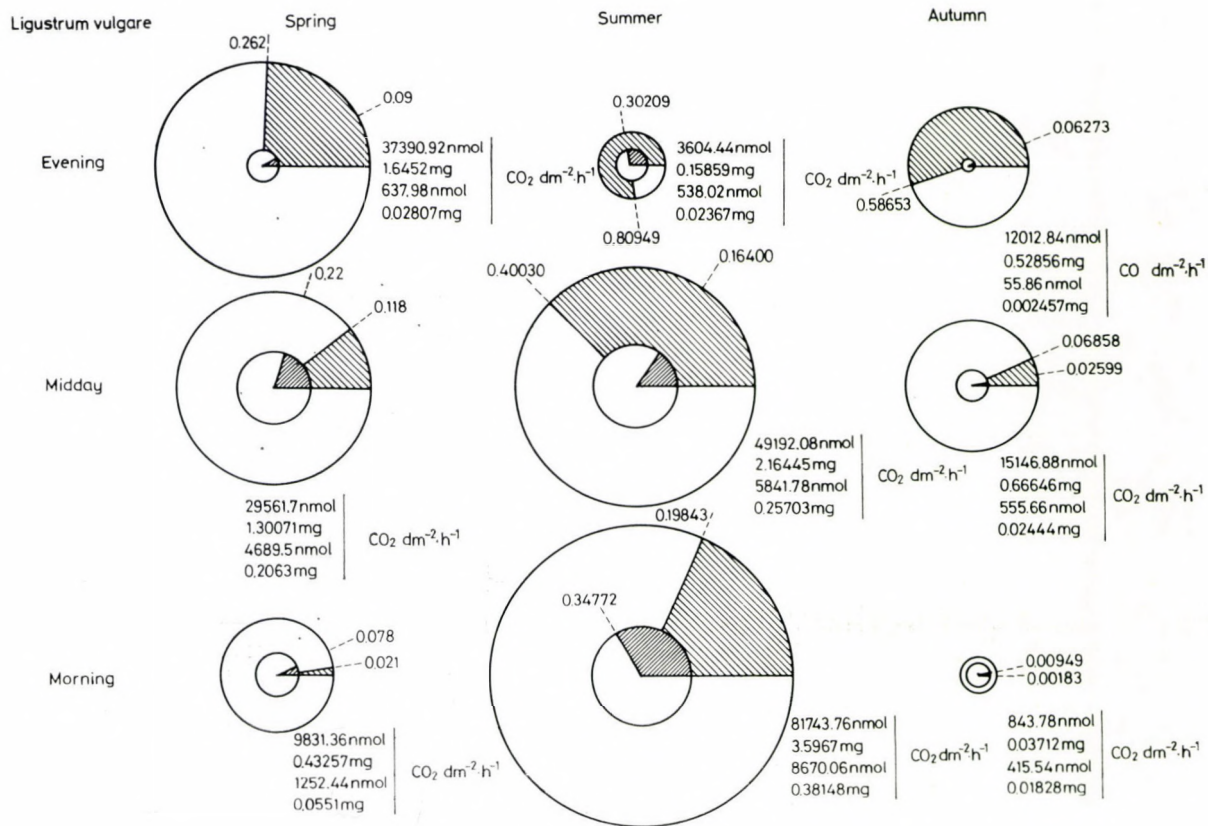


Fig. 3. CO₂ fixation values in *Ligustrum vulgare*

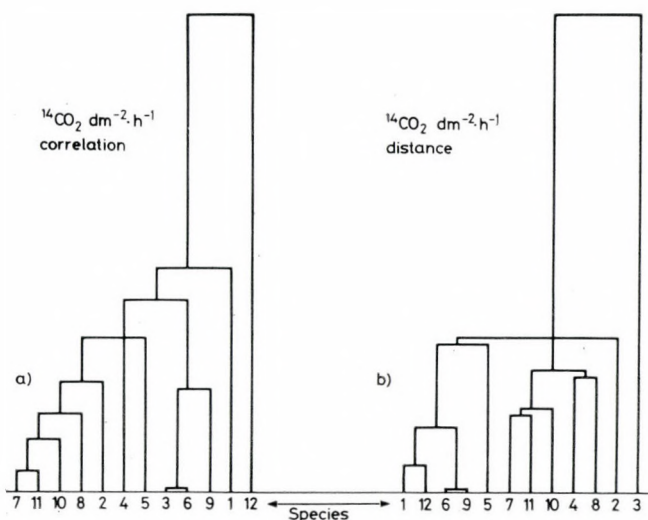


Fig. 4. Cluster diagrams of relations between the photosynthetic characteristics of the species.
A: by correlation, B: by distance

Table 4

Percentage distribution of photosynthetic intensity over the day in relation with the available light energy, on the basis of all data of measuring

| Part of the day | Intensity of photosynthesis | | | |
|-----------------|-----------------------------|--------------|-------|-------|
| | Herb stratum | Tree stratum | Total | Joule |
| | % | | | |
| Morning | 5.85 | 27.77 | 33.62 | 62.01 |
| Noon | 5.87 | 39.5 | 45.37 | 30.25 |
| Evening | 1.05 | 19.95 | 21.01 | 7.73 |

Table 5

Ratios of photosynthetic intensity and light energy in the herb- and tree stratum

| Part of the day | Photosynthetic intensity | Light energy (Joule) |
|-----------------|-----------------------------|-----------------------------|
| | Herb stratum : Tree stratum | Herb stratum : Tree stratum |
| Morning | 17 : 83 | 5.95 : 94.05 |
| Noon | 13 : 87 | 11.66 : 88.34 |
| Evening | 5 : 95 | 5.53 : 94.47 |

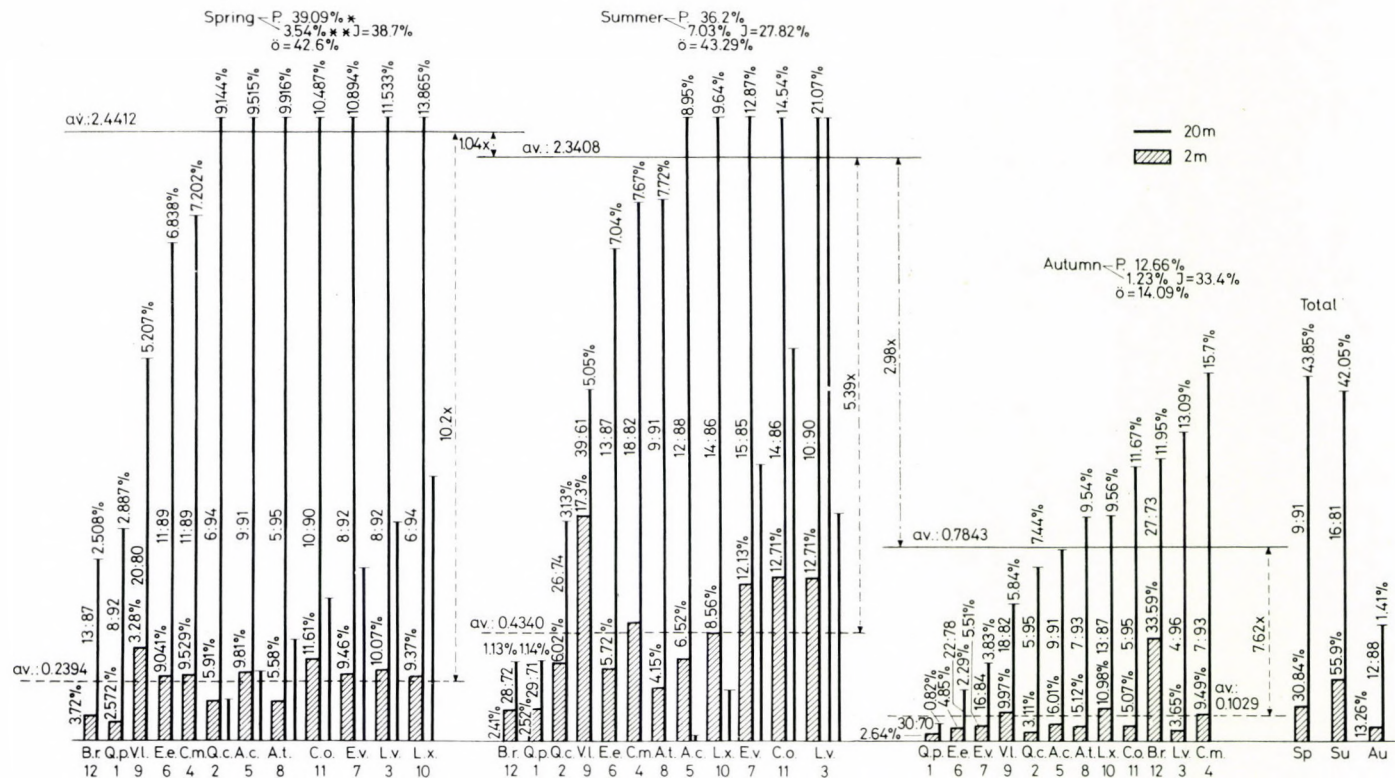


Fig. 5. Photosynthetic intensities of the species in the different seasons. P = photosynthetic intensity, * 20 m, ** 2 m

When measured in summer CO_2 fixation was only one and a half times higher in direct light than in shade.

The percentage distribution of photosynthetic intensity over the day relative to the values of the available light energy is shown in Table 4.

As seen from the table the efficiency of photosynthesis is highest in the evening and lowest in the morning.

Table 5 shows the ratio of productivity to the available light energy (Joule) in the herb- and tree stratum.

In the tree stratum the utilization of light is poorer in the morning than in the evening. In the herb stratum it is the other way round.

The utilization of light energy i.e. the efficiency values for the different species are represented in Fig. 6. The lowest efficiency (0.071%) was found in *Quercus petraea*, the highest (0.4%) in *Ligustrum vulgare*. The other species can be placed in two categories:

I. $E = 0.153-0.197\%$

Bromus ramosus
Quercus cerris
Euonymus europaeus
Acer tataricum
Acer campestre

II. $0.277-0.332\%$

Lonicera xylosteum
Euonymus verrucosus
Viburnum lantana
Crataegus oxyacantha
Cornus mas

In Fig. 7 the values of photosynthetic efficiency in the tree- and herb strata of the species are seen. In spite of a tenfold difference in light energy there is not much difference in average efficiency between the two strata. However, with the species evaluated one by one, considerable differences are found:

The efficiency increased:

I. In shade

| | Tree stratum, Herb stratum, | |
|-------------------------|-----------------------------|-------|
| | % | % |
| <i>Quercus petraea</i> | 0.06 | 0.082 |
| <i>Bromus ramosus</i> | 0.08 | 0.21 |
| <i>Viburnum lantana</i> | 0.26 | 0.38 |

II. In direct light

| | Herb stratum, Tree stratum, | |
|-----------------------------|-----------------------------|------|
| | % | % |
| <i>Quercus cerris</i> | 0.14 | 0.25 |
| <i>Acer tataricum</i> | 0.16 | 0.20 |
| <i>Acer campestre</i> | 0.16 | 0.23 |
| <i>Lonicera xylosteum</i> | 0.21 | 0.34 |
| <i>Euonymus verrucosus</i> | 0.22 | 0.34 |
| <i>Cornus mas</i> | 0.25 | 0.41 |
| <i>Crataegus oxyacantha</i> | 0.26 | 0.38 |
| <i>Ligustrum vulgare</i> | 0.27 | 0.52 |

The high rate increase of photosynthetic efficiency in *Viburnum lantana* and *Bromus ramosus* under poorer light conditions suggests their shade loving nature. The photosynthetic efficiency of *Ligustrum vulgare* in strong light is nearly twice as high as in shade, which is connected with its expressed heliophilous character.

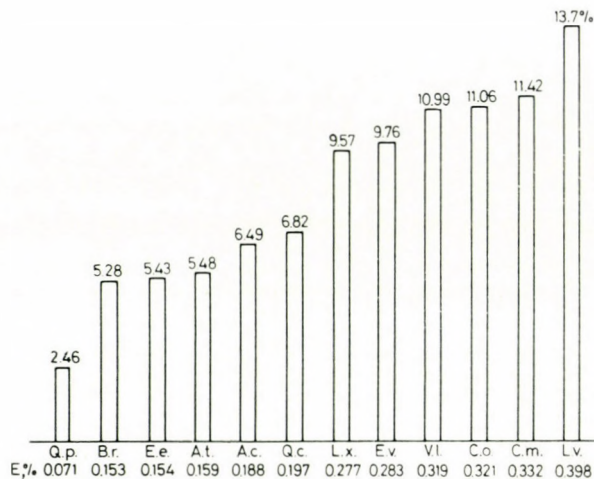


Fig. 6. Percentage distribution of photosynthetic efficiency values among the species

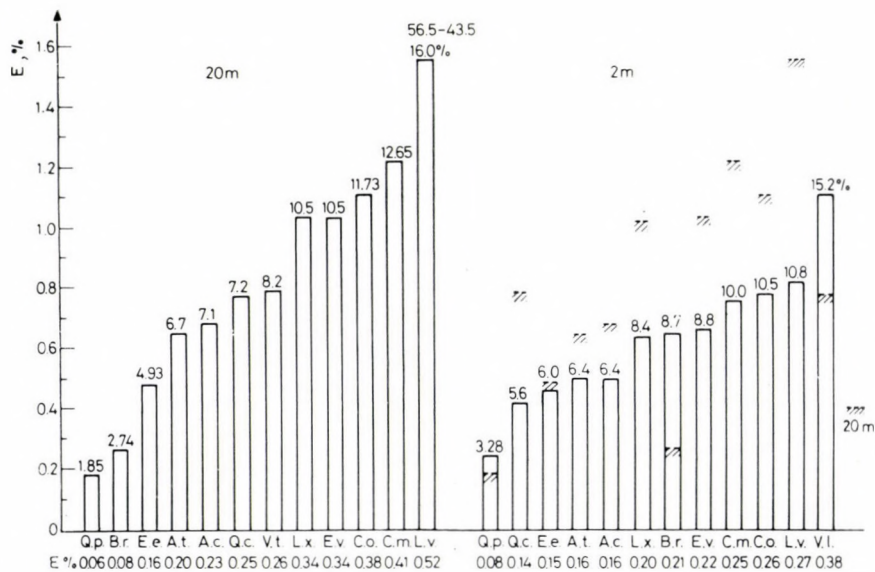


Fig. 7. Photosynthetic efficiency values of the species in herb stratum (2 m) and in tree stratum (20 m)

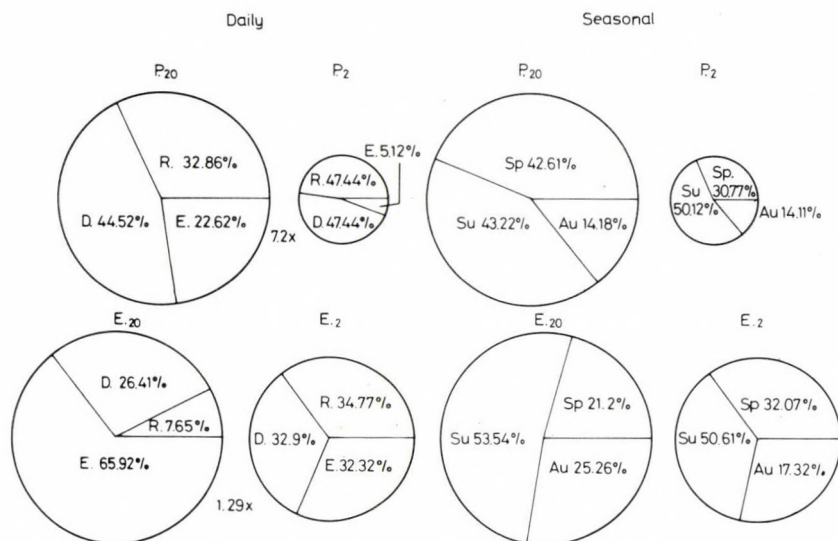


Fig. 8. Percentage distribution of photosynthetic productivity (P) and efficiency (E) by season and part of the day on the basis of all data of measuring. The areas of the circles are proportionate to the values obtained in the herb stratum (2 m) and tree stratum (20 m). R = morning, D = noon, E = evening, S = spring, Su = summer, A = autumn

Figure 8 shows the percentage distribution of photosynthetic intensity and efficiency values by part of the day, season and stratum on the basis of all data of measuring. The parts of the day are characterized by a gradual increase of efficiency—from 0.18 to 0.53% on the average—in the tree stratum from morning till night. This tendency is not found in the herb stratum which showed an average efficiency value of about 0.2% during the day. As for the seasons: the highest photosynthetic efficiency was measured in summer, 0.32% in the herb stratum and 0.42% in the tree stratum. The autumn utilization of light energy was similar to that in spring, 0.1–0.2 per cent on an average.

Figure 9 represents the average values of photosynthetic efficiency. It can be seen that the efficiency increased from morning till night, with the difference that in summer the herb stratum showed the highest photosynthetic efficiency in the morning. The measuring data are widest scattered in the morning and come closer to one another at noon. The latter was most characteristic of *Lonicera xylosteum*, *Acer tataricum* and *Cornus mas*. In autumn, and particularly in the morning the efficiency values were very low in the tree stratum because of the low temperatures (7–7.5 °C).

Acer tataricum, *Quercus petraea* and *Euonymus verrucosus* were highly responsive to low temperatures ($E = 0.0027$, 0.0025 and 0.003% , respectively). Less so was *Bromus ramosus* ($E = 0.068\%$) which attained the highest efficiency values in autumn in the herb stratum (Fig. 10). Maximum efficiency was displayed by most species in summer evening in the tree stratum; the highest value—1.54%—was found in *Ligustrum* (Fig. 11).

The space lattice diagram (Fig. 12) shows the relation of photosynthetic intensity with light energy and temperature. It is clearly seen that when the temperature falls below 18 °C the intensity of photosynthesis gradually decreases, and the decrease considerably

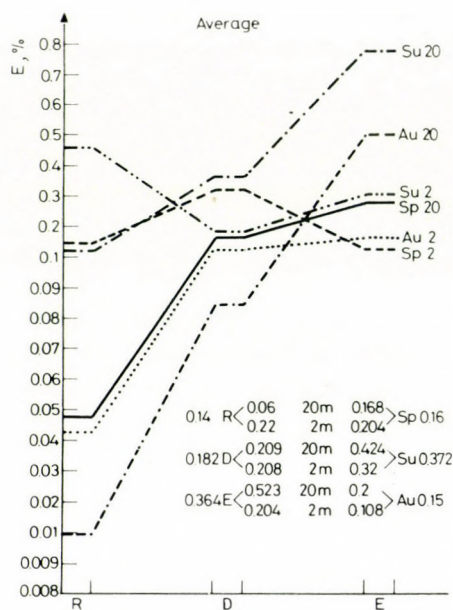


Fig. 9. Efficiency values on the average of the species ($E\%$) by part of the day and season
 2 m = herb stratum, 20 m = tree stratum; R = morning, D = noon, E = evening; S = spring, Su = summer, A = autumn)

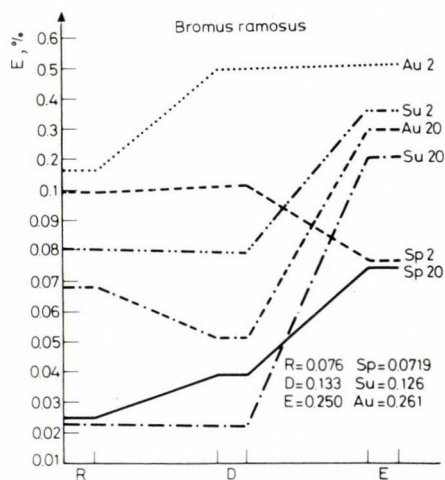


Fig. 10. Efficiency values in *Bromus ramosus* ($E\%$)

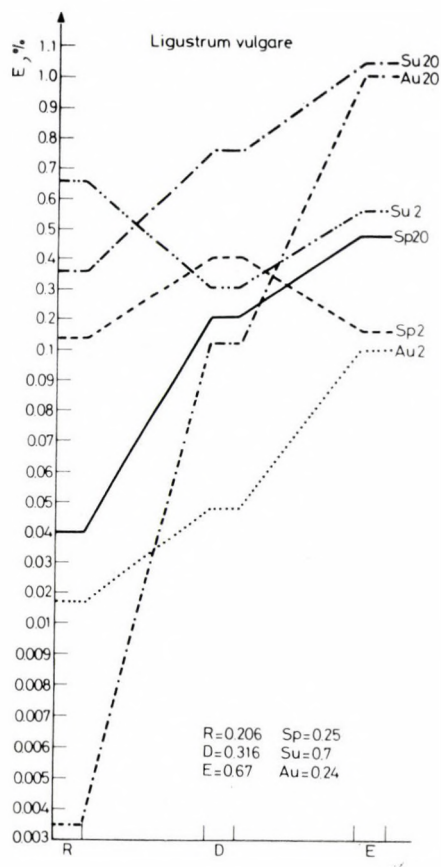


Fig. 11. Efficiency values in *Ligustrum vulgare* (E%)

accelerates below 9 °C. With the light energy reduced to less than about 30 Joule the photosynthetic activity slows down.

Some production data calculated from the values of CO₂ fixation:

Photosynthetic production values of the vegetation period:

| | |
|-----------------------------------|---|
| in the tree stratum | 150 g · m ⁻² , LAI value 208.6 g · m ⁻² |
| (average of the species) | |
| in the herb stratum | 22 g · m ⁻² , LAI value 31.4 g · m ⁻² |
| (average of the species) | |
| <i>Ligustrum vulgare</i> average: | 158.7 g CO ₂ · m ⁻² |
| maximum: | 891.0 g CO ₂ · m ⁻² |
| average of 1 shrub: | 56.8 g CO ₂ · m ⁻² |
| <i>Quercus petraea</i> average: | 20.9 g CO ₂ · m ⁻² |
| maximum: | 178.8 g CO ₂ · m ⁻² |

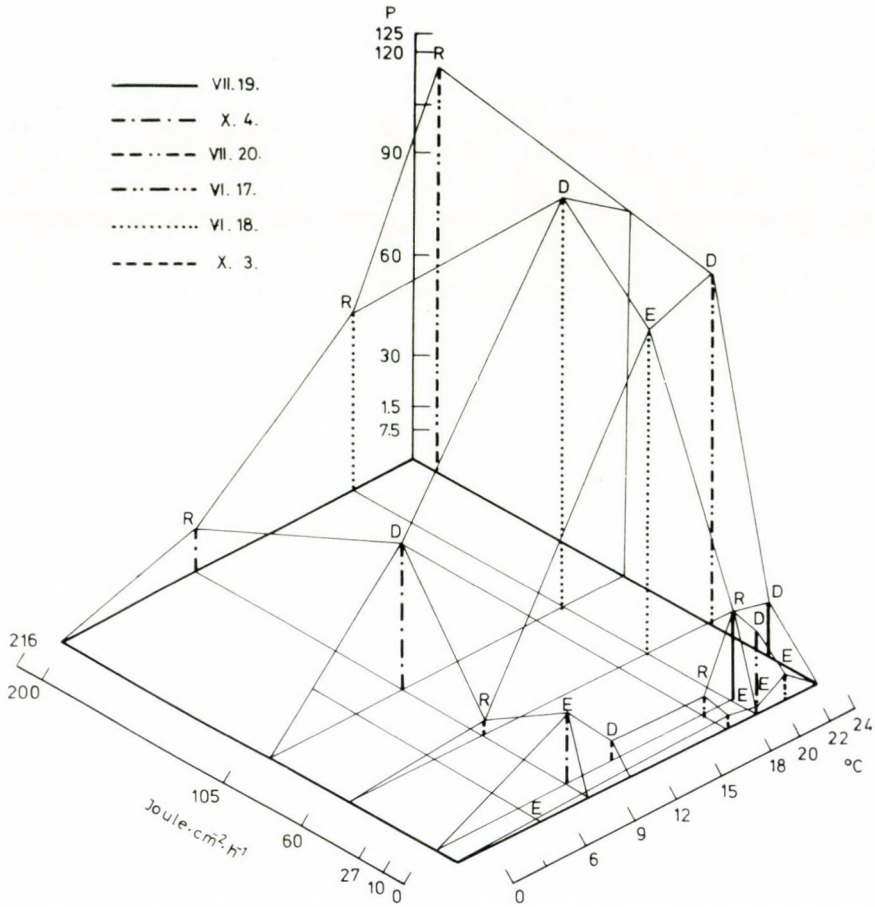


Fig. 12. Values of photosynthetic productivity (P) in relation with temperature and light energy (A P = relative values of radioactivity in leaves)

Discussion

According to the results of investigations the species examined are characterized by the following:

- The photosynthetic activity of *Quercus petraea* was found to be of low intensity on each occasion of measuring. This statement is supported by the production data of the table compiled by H. W. ART and P. L. MARKS (1971) too. The low photosynthetic productivity is supposed to be related with the anatomy of the oak leaf which greatly increases the diffusion resistance (SUBA et al. 1982).

- *Quercus cerris* showed in most cases a higher photosynthetic productivity than *Qu. petraea*.
- At high intensities of light the photosynthetic activity increased most of all in *Ligustrum vulgare*, *Crataegus oxyacantha*, *Lonicera xylosteum* and *Euonymus verrucosus*, and to a somewhat lesser extent in *Acer tataricum*, *Acer campestre* and *Cornus mas*.
- *Viburnum lantana* made a much better use of a poorer light than the other species; e.g. in summer, measuring in the morning in the shaded herb stratum pointed out a higher rate of CO_2 fixation ($0.673 \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{h}^{-1}$) in it than in species with the highest photosynthetic intensity.
- *Acer tataricum* was more responsive than the other species to poor light conditions and low temperatures; under such conditions its photosynthetic activity was sharply reduced.
- *Bromus ramosus* occupies a peculiar place among the species examined. Its photosynthesis generally is characterized by low values, it does not make much use of strong light, in summer its production was higher when in shade than when in direct light. In autumn its production increased 3.47-fold compared to the summer performance.
- The species generally were characterized by a very low photosynthetic activity in autumn, even in intensive light, owing to the low temperature ($7\text{--}7.5^\circ\text{C}$). There was hardly any difference in photosynthetic intensity between the shaded herb stratum and the tree stratum, although in light energy the difference was 42-fold. In cold autumn mornings the species fixed three times less CO_2 with a double light energy supply than at noon. *Acer tataricum* was the most sensitive to cold. The photosynthesis of *Bromus ramosus* was hardly—if at all—affected by the cold, which indicates that the heat optimum of its photosynthesis is low. The same applies, though to a lesser extent, to *Lonicera xylosteum*.
- In autumn the lowest values of photosynthetic intensity were found in the two *Euonymus* species. In comparison to the summer results of measuring *Euonymus verrucosus* showed a 9.8-fold, *E. europaeus* an 8.1-fold decrease of photosynthetic intensity compared to the three-fold average.
- With all measuring data taken into consideration, the largest amount of CO_2 ($3.5 \text{ mg} \cdot \text{dm}^{-2} \cdot \text{h}^{-1}$) was fixed by *Ligustrum vulgare* on 20 July in the morning, at a light intensity of 20.030 Joule and temperature of 23.2°C [HEINICKE (1966) obtained a net photosynthesis value of $7.4 \text{ mg CO}_2 \cdot \text{h}^{-1}$ with apple tree.] At that time *Crataegus oxyacantha* also fixed much CO_2 ($2.315 \text{ mg dm}^{-2} \cdot \text{h}^{-1}$). As a contrast to the former species only 0.207 mg was measured in *Quercus petraea* and 0.235 mg in *Bromus ramosus*. The widest difference is thus 17.3-fold.

The percentage values of efficiency established by us come close to those published by G. M. VAN DYNE et al. (1975) as average values of various

vegetation types. According to LARCHER (1980) in the natural plant associations the efficiency is below 1%. With the exception of *Quercus petraea*, *Bromus ramosus* and *Viburnum lantana* the value of efficiency in the species examined increased in stronger light, which indicates their heliophilous nature. The increase was of particularly great extent in *Ligustrum vulgare* and *Cornus mas*. The utilization of light energy was most efficient under poorer light conditions. This conforms to the statement of HARTT and KORTSCHAK (1967) and SHAGINA (1955) who point out that under the poor evening light conditions the light energy absorbed is used first of all in the photosynthesis rather than in other processes demanding energy, as e.g. the material transport.

The summer maxima of the photosynthetic intensity and efficiency values may be related with the development stage and metabolism of plant and leaf, respectively, and with the environmental factors (SAEKI and NOMOTO 1958, PISEK and WINKLER 1958).

The space lattice diagram that shows the influence of ecological factors on the intensity of photosynthesis expresses well the hereditary features of plants and their adaptation to the site conditions. Similar relationships are suggested by a number of authors (LARCHER 1969, 1973, 1980; MOONEY et al. 1978; SAWADA and MIYACHI 1974; STRAIN et al. 1975; BERRY and BJÖRK-MANN 1980).

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EFFECT OF DICAMBA ON THE HISTOLOGICAL STRUCTURE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) LEAVES

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Changes taking place in the tissues of sunflower leaves in response to three concentrations of dicamba (2-methoxy-3,6-dichlorobenzoic acid) applied at three different times were studied. With a view to evaluating the effect, the thickness of the leaf blade, the length of the palisade and spongy parenchyma cells and the wall thickness of the epidermis cells on the upper and lower leaf surfaces were measured; the number of cell rows in the palisade and spongy parenchyma were counted; and the distances between the vascular bundles were established. By the time the third sample was taken all three concentrations caused a thickening of the leaf blade compared to the control. In comparison to the effects of 100 and 5000 ppm concentrations the 1000 ppm concentration caused a very great extent of thickening. The treated plants differ considerably from the control as regards the length of the cells in the palisade parenchyma of the leaf: in every case the cells are much shorter. The effect of 1000 ppm is again noteworthy, since this concentration produces the sharpest reduction in the length of the cells. The cells of the spongy parenchyma are also shorter than the corresponding cells in the control leaves. Dicamba applied at a concentration of 1000 ppm caused a slight lengthening of these cells, so there was no longer any great difference in length between the palisade and spongy parenchyma cells. The walls of the epidermis cells on both sides of the leaf became noticeably thinner compared to the control in response to all three concentrations. On the basis of the data it can be established that the greatest changes in the tissue structure of sunflower leaves were caused by the 1000 ppm concentration of dicamba; higher concentrations no longer resulted in any considerable increase in the damage.

Introduction

Agriculture uses large volumes of hormone-based herbicides, particularly for the chemical control of cereals. These herbicides are mostly sprayed from aeroplanes or helicopters, so they may drift and cause damage and destruction to dicotyledonous crops, which are particularly sensitive to hormone-based herbicides. These herbicides cause deformations in both the aboveground and underground parts of the plants (UBRIZSY and GIMESI 1969). In the aboveground part of the plant distortion and twisting of the shoots, funnel-shaped leaves (BUHL 1958, UBRIZSY 1962), interlacing of the leaves (WAY 1962, 1963b, 1964a, KIERMAYER cit. AUDUS 1964), fanleaf and ginkgo-leaf in vines (UBRIZSY 1962, SZATALA 1967) and other morphological changes (ANDERSEN, BACHTHALLER, HANF cit. UBRIZSY 1962, TERPÓ-POMOGYI and TERPÓ 1971) are found. WAY (1963a, 1963c, 1964b) also observed the deformation of the leaves. Considerable distortions are caused by hormone-based herbicides in the fruit (UBRIZSY 1962), and severe damage is also found in the roots (BUHL 1958, WAY 1962a, 1963b, 1964a).

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The high sensitivity of sunflower is mentioned by UBRIZSY (1962). According to WATSON (1948) serious damage is caused by hormone-based herbicides during cell differentiation; much less harm is done to the leaf if it is fully or almost fully developed at the time of the treatment. In the meristematic tissues and the phloem parenchyma of the stalk a high rate of cell multiplication occurs under the influence of hormone-based herbicides (EAMES 1951). These herbicides have a considerable influence on cell-wall formation as well (GORTER, GIFFORD cit. AUDUS 1964).

The mesophyllous cells became narrow and of irregular shape as a response to treatments with 2,4-D (TUKEY et al. 1945). Cells of the palisade parenchyma increase in size in all directions, but particularly longitudinally (WATSON 1948). The elongation of the palisade parenchyma cells results in a thickening of the leaf (BRADLEY et al. cit. AUDUS 1976).

HACCIUS (cit. AUDUS 1964) observed deformation of the spongy parenchyma cells as a consequence of treatments with hormone-based herbicides. On the abaxial surface of the leaf the veins protrude conspicuously (FELBER 1948, WATSON 1948). The vascular bundles in the leaf are placed close to one another (EAMES 1949, WATSON 1948).

In the case of wheat and barley dicamba had an effect similar to that of 2,4-D (FRIESEN et al. 1964). PATE et al. (1965) found lesions in the phloem, cambium and the adjacent parenchyma in plants treated with dichlobenil and dicamba.

Material and method

Sunflower plants (variety: VNIIMK 6541) were used in the experiment. Sowing was carried out on 12th May; the plants were treated on 12th June, on reaching a height of 12 cm.

The herbicide applied was dicamba (2-methoxy-3,6-dichlorobenzoic acid) of technical grade.

The herbicide was applied onto the surface of the plants by spraying.

The treatments were:

| | |
|---------|----------|
| dicamba | 100 ppm |
| dicamba | 1000 ppm |
| dicamba | 5000 ppm |
| control | |

Leaf samples were collected on three occasions:

| | |
|------------|-----------|
| 1st sample | 14th June |
| 2nd sample | 21st June |
| 3rd sample | 11th July |

On all three occasions the samples were taken in the morning, then fixed in 40% alcohol. Excisions were made from the middle of the leaf by means of an MC-2 slide microtome fitted with a KTOC-2 electric freezer. The sections were stained with Ehrlich's acidic haematoxylin. The experiment was statistically evaluated. The thickness of the leaf blade, the thickness of the epidermis cell-walls on the adaxial surfaces of the leaf and the lengths of the palisade and spongy parenchyma cells were measured. In the case of spongy parenchyma cells, the term length means the distance between the two farthest points of the cells.

The data were evaluated by the double *t*-test. The number of cell rows in the palisade and spongy parenchyma was also counted, and the distance between the vascular bundles in the leaves was measured.

Results

Tissue lesions caused by dicamba are shown in Figs 1–3.

Examination of the control leaf

Sample 1 (taken on 14th June). The leaf is bifacial (Fig. 4A); stomata are found in both the upper and lower epidermis of the leaf. The palisade parenchyma consists of 2 rows of elongated cells not closely set (Fig. 6A). The length of the cells is 2–3 times their width. The spongy parenchyma is 2–3 cell-rows thick; the loosely arranged cells are of irregular shape (Fig. 6G). The leaf protrudes slightly along the major leaf veins. The vascular bundles are widely spaced, at an average distance of $113\text{ }\mu\text{m}$ ($88\text{--}139\text{ }\mu\text{m}$) from one another.

Sample 2 (taken on 21st June). The arrangement of the palisade and spongy parenchyma cells is similar to that in Sample 1 (Fig. 4B). The palisade parenchyma is 2 cell-rows thick and the spongy parenchyma 2–3 cell-rows thick (Fig. 6H). The cells of the palisade parenchyma are slightly elongated (Fig. 6B), their length being 3–4 times their width. The vascular bundles are arranged in a manner similar to that observed in Sample 1; the average distance between them is $119\text{ }\mu\text{m}$ ($98\text{--}140\text{ }\mu\text{m}$). The leaf blade is uniformly thick, no protrusions or depressions being found on either the upper or the lower surface of the leaf.

Sample 3 (taken on 11th July). The cells of the palisade parenchyma are arranged in 2 cell-rows (Fig. 6C) and those of the spongy parenchyma in 2–3 cell-rows (Fig. 6I), in much the same way as in Sample 1 (Fig. 4C). The cells of the palisade parenchyma are 4–5 times

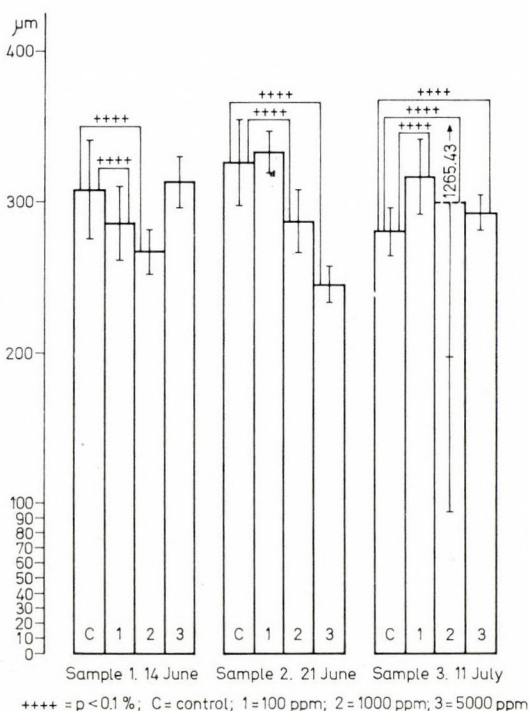


Fig. 1. Changes in the thickness of the leaf in response to treatments with dicamba

longer than they are wide. The arrangement of the palisade and spongy parenchyma cells and of the vascular bundles is similar to that in Sample 1. The vascular bundles are at an average distance of $107\ \mu\text{m}$ ($82\text{--}132\ \mu\text{m}$) from each other. The leaf blade is of uniform thickness; the epidermis on both sides is similar to that of Sample 1.

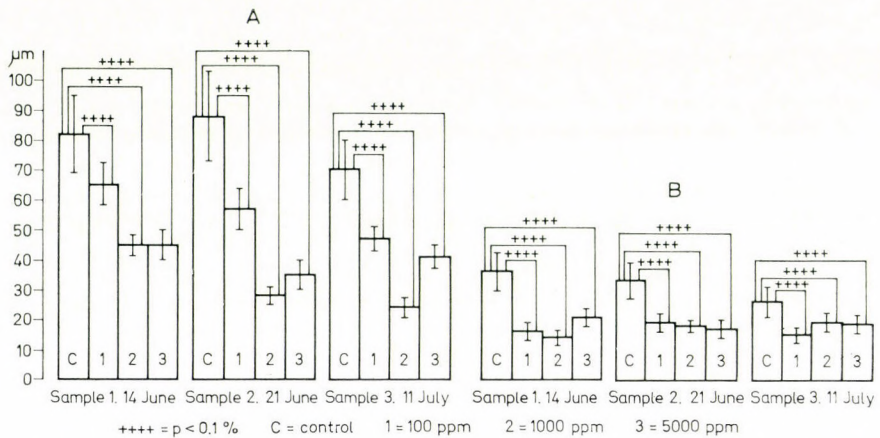


Fig. 2. Changes in the length of palisade parenchyma cells in response to treatments with dicamba (a). Changes in the length of spongy parenchyma cells in response to treatments with dicamba (b)

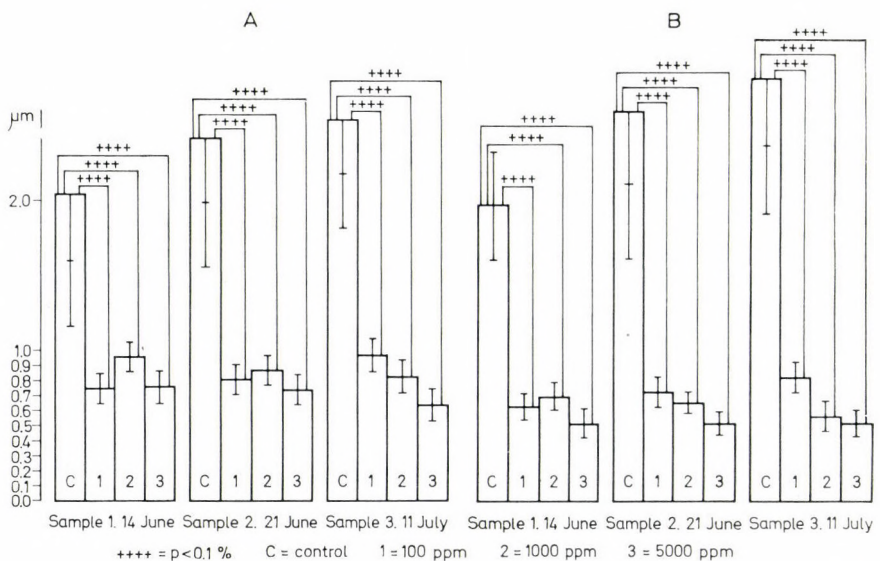


Fig. 3. Changes in the thickness of epidermis cell-walls on the upper leaf surface as a result of treatment with dicamba (a). Changes in the thickness of cell-walls in the lower leaf epidermis (b)

Structure of leaf treated with dicamba

Effect of 100 ppm dicamba

Sample 1 (14th June). The palisade parenchyma (Fig. 6D) is 1–2 cell-rows wide; cells towards the centre of the mesophyll are shorter, their length being once or twice their width, while those immediately below the epidermis are 2–3 times longer than they are wide. The cells are not close set. The spongy parenchyma cells (Fig. 6K) are roundish, oval or occasionally of irregular shape, arranged loosely in 3–4 cell-rows. The vascular bundles are much closer to one another than in the control, the average distance between them being $32\ \mu\text{m}$ ($27\text{--}37\ \mu\text{m}$). The leaf blade is of uniform thickness (Fig. 4D); the epidermis on both sides of the leaf is free from protrusions and depressions. On the abaxial surface of the leaf the veins protrude slightly.

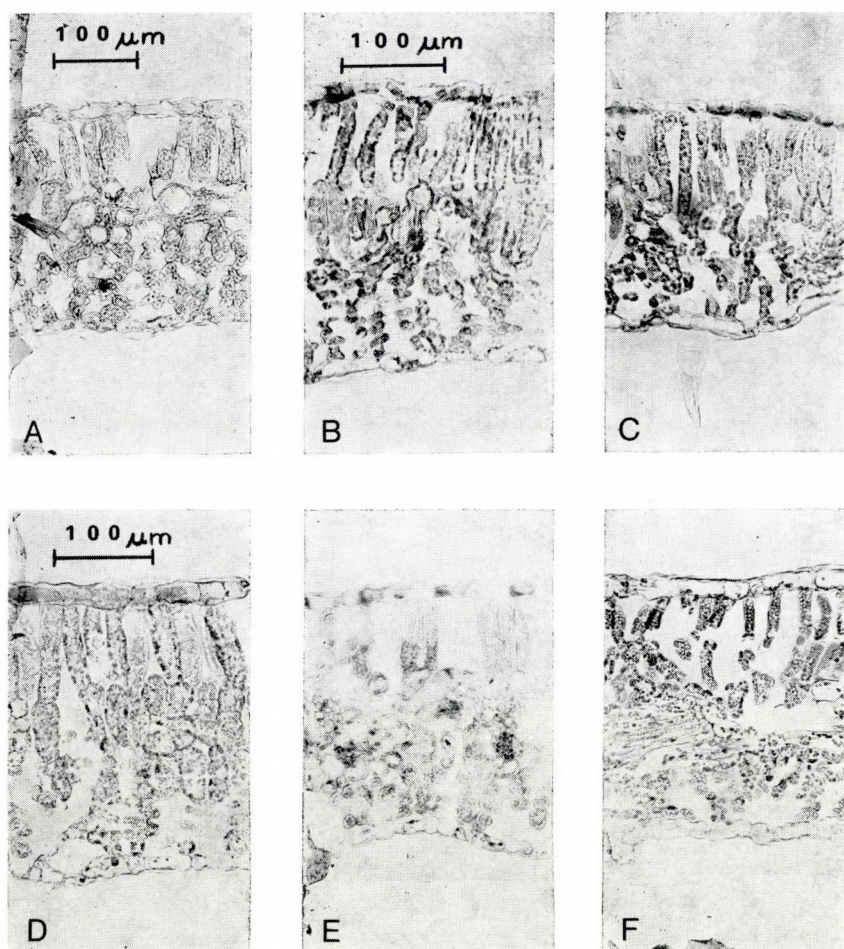


Fig. 4. Cross-sections of leaves in the control and in plants treated with 100 ppm dicamba. — A–C = control; D–F = 100 ppm; A, D = Sample 1 (14th June); B, E = Sample 2 (21st June); C, F = Sample 3 (11th July). — Figs B, C, E and F are to the same scale

The thickness of the leaf is greatly reduced compared to the control (Fig. 1). A considerable reduction in length is found in the cells of the palisade parenchyma, and even more so in those of the spongy parenchyma (Fig. 2). The thickness of the epidermis cell-walls on both the upper and lower leaf surfaces is extremely reduced compared to the control (Fig. 3).

Sample 2 (21st June). The palisade parenchyma cells are arranged in 1–2 cell-rows (Fig. 6E); the cells below the epidermis are 2–4 times longer, and those towards the mesophyll are 2–3 times longer than they are wide. The cells are much closer set than in Sample 1. The roundish, oval or irregularly shaped cells of the spongy parenchyma (Fig. 6L) form a layer 3–5 cell-rows wide; they are slightly closer set than those of Sample 1. The epidermis of the upper leaf surface is free of protrusions and depressions, while on the lower leaf surface they are found in fairly large numbers (Fig. 4E). On the lower leaf surface the leaf veins protrude to a considerable extent.

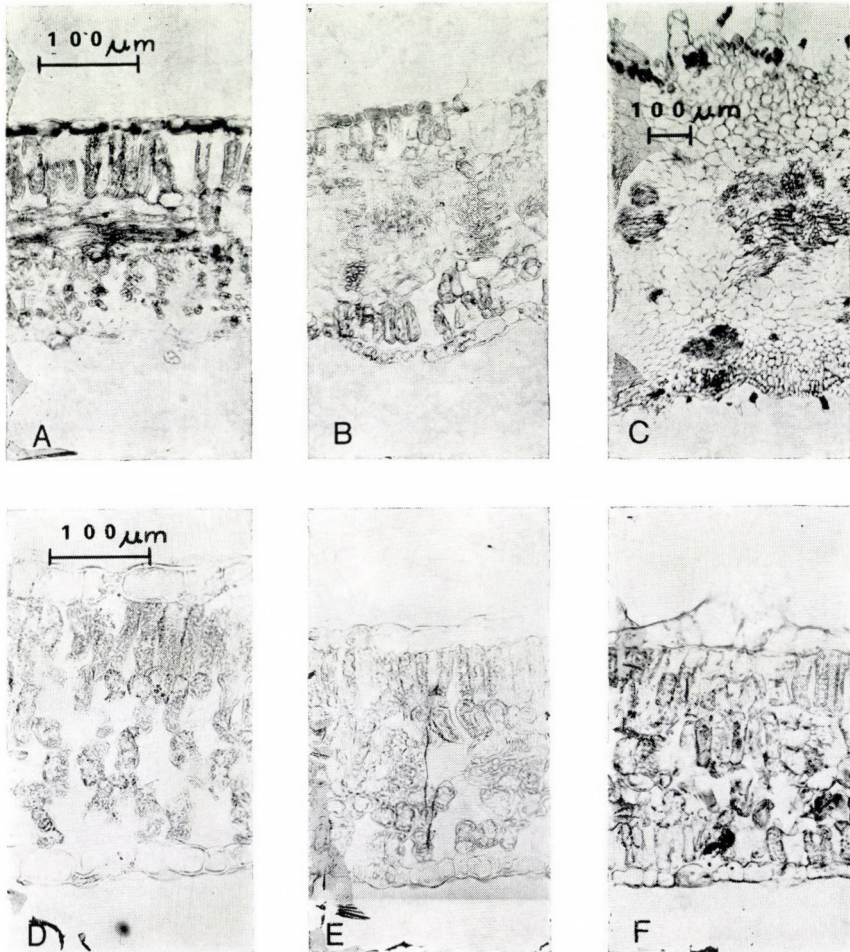


Fig. 5. Cross-sections of leaves from plants treated with 1000 and 5000 ppm dicamba. — A–C = 1000 ppm; D–F = 5000 ppm; A, D = Sample 1 (14th June); B, E = Sample 2 (21st June); C, F = Sample 3 (11th July). — Figs A, B and F are to the same scale, as are Figs D and E

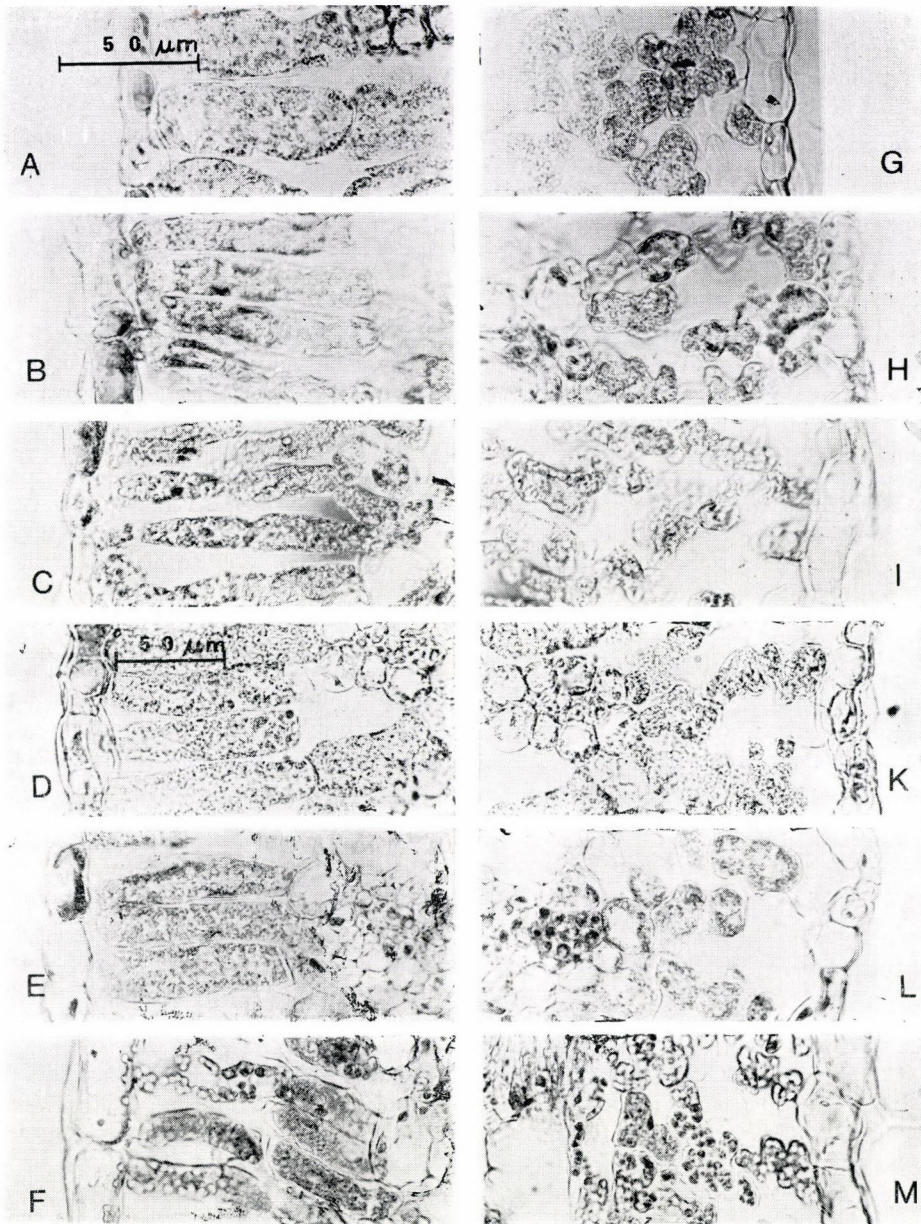


Fig. 6. Palisade and spongy parenchyma cells in the leaves of the control and of plants treated with 100 ppm dicamba. — A—F = palisade parenchyma cells; A—C = control; D—F = 100 ppm; G—M = spongy parenchyma cells; G—I = control; K—M = 100 ppm; A, D, G, K = Sample 1 (14th June); B, E, H, L = Sample 2 (21st June); C, F, I, M = Sample 3 (11th July). — Figs A, B, C, E, F, G, H, I, L and M are to the same scale, as are Figs D and K

The vascular bundles occupy one-third of the thickness of the leaf blade; they are located close to one another, the distance between them ranging from 16–24 μm .

In comparison to Sample 1 the leaf blade has thickened and reached a thickness similar to that of the control (Fig. 1).

The cells of the palisade parenchyma are similar in length to the cells of Sample 1, considerably shorter than in the control (Fig. 2). The length of the spongy parenchyma cells has slightly increased compared to Sample 1 (Fig. 2), but are much shorter than those in the control.

Although the thickness of the cell-walls in the epidermis is very slightly increased on both sides of the leaf compared to Sample 1; it is substantially reduced compared to the control (Fig. 3).

Sample 3 (11th July). The palisade parenchyma consists of 1–2 rows of cells which are not closely set (Fig. 6F); cells towards the centre of the mesophyll are 1–2 times longer, and those towards the epidermis are 3–4 times longer than they are wide. The spongy parenchyma consists of 3–4 rows of roundish, oval or irregularly shaped cells (Fig. 6M). The cells are loosely arranged in the rows. The thickness of the leaf blade is not uniform (Fig. 4F). The epidermis on the upper leaf surface is free of protrusions and depressions; the lower surface, however, is rather undulatory with a noticeable protrusion of the leaf veins. Some one-third of the thickness of the leaf blade is occupied by the vascular bundles, which are placed close to one another, at an average distance of 36 μm (24–46 μm). The leaf blade is somewhat thinner than in Sample 1 (Fig. 1), but in comparison to the control it has become considerably thicker. The edges of the leaf have thickened about two-fold compared to the section towards the middle of the leaf; here the closely spaced vascular bundles take up more than half the thickness of the leaf. The cells of the palisade parenchyma (Fig. 2) show a considerable reduction in length compared to both the previous samples and the control. The cells of the spongy parenchyma (Fig. 2) are also shorter than in either the previous samples or in the control. Although the cell-walls in the epidermis (Fig. 3) are slightly thicker on both leaf surfaces than in the previous samples, they are very thin compared to the control.

Effect of 1000 ppm dicamba

Sample 1 (14th June). The cells of the palisade parenchyma (Fig. 7A) are arranged in 1–2 cell-rows. The length of cells near the epidermis is 2–3 times their width, while cells towards the centre of the mesophyll are 1–2 times longer than they are wide. The cells are arranged fairly close to one another.

The spongy parenchyma (Fig. 7G) consists of 3–5 rows of loosely arranged cells. The cells are roundish or oval, and occasionally of irregular shape.

The thickness of the leaf blade is fairly uniform (Fig. 5A); on the upper leaf epidermis there are no protrusions or depressions, but the epidermis of the lower leaf surface is slightly undulatory. The leaf veins on the abaxial surface show a considerable extent of protrusion. The vascular bundles occupy one-third of the thickness of the leaf; they are placed at an average distance of 28 μm (27–30 μm) from each other.

The thickness of the leaf blade (Fig. 1) is greatly reduced compared to both the control and Sample 1 of the 100 ppm treatment. The cells of the palisade parenchyma (Fig. 2) are much shorter than either in the control or in Sample 1 of the 100 ppm treatment. The cells of the spongy parenchyma have only become noticeably shorter (Fig. 2) compared to the control; in comparison to Sample 1 of the 100 ppm treatment there is hardly any difference. The walls of the epidermis cells are very thin compared to the control on the upper and lower leaf surfaces alike (Fig. 3); however, in comparison with Sample 1 of the 100 ppm treatment a certain extent of thickening can be observed.

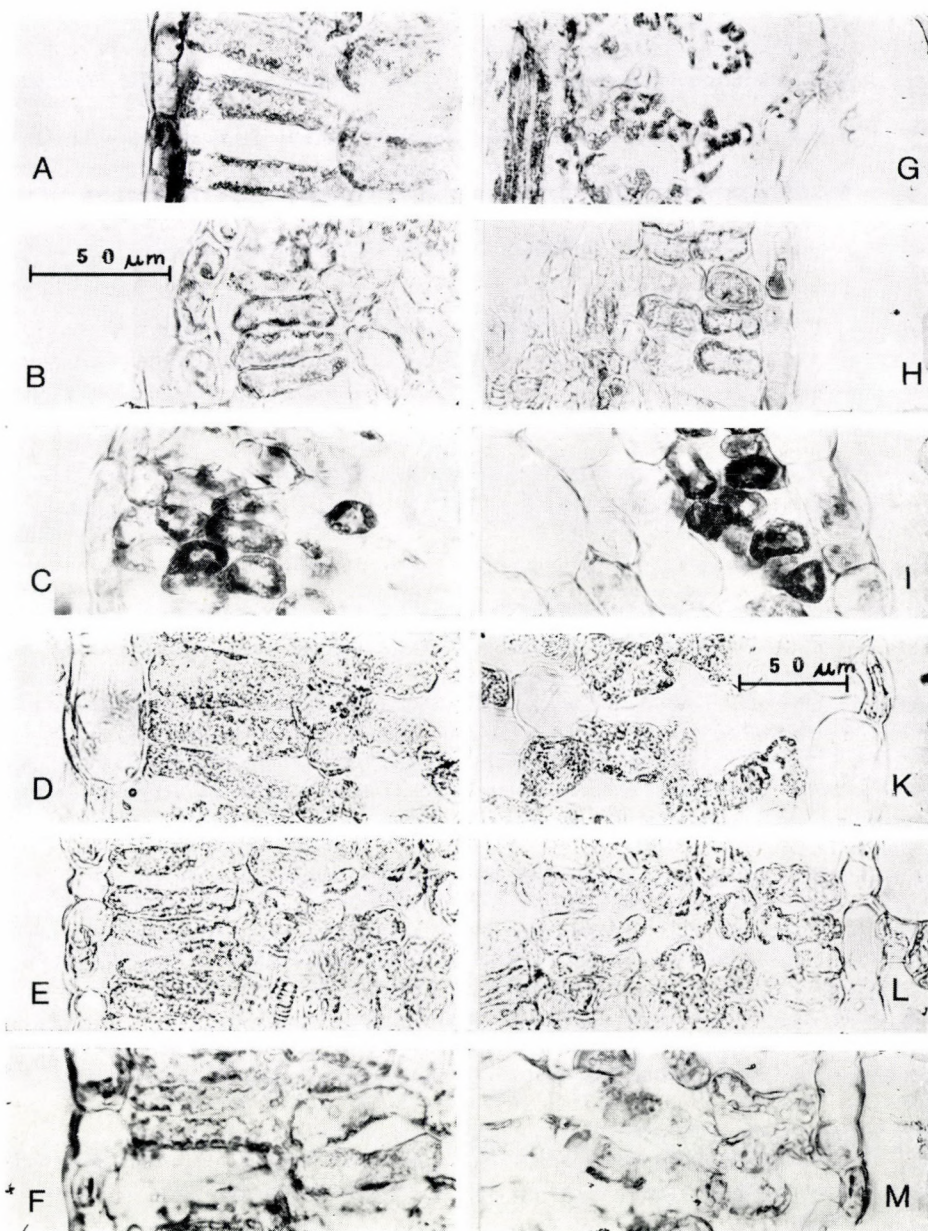


Fig. 7. Palisade and spongy parenchyma cells in the leaves of plants treated with 1000 and 5000 ppm dicamba. — A—F = palisade parenchyma cells; A—C = 1000 ppm; D—F = 5000 ppm; G—M = spongy parenchyma cells; G—I = 1000 ppm; K—M = 5000 ppm; A, D, G, K = Sample 1 (14th June); B, E, H, L = Sample 2 (21st June); C, F, I, M = Sample 3 (11th July). — Figs A, B, C, F, G, H, I and M are to the same scale, as are Figs D, E, K and L

Sample 2 (21st June). The palisade parenchyma is 1–2 cell-rows wide (Fig. 7B). The cells are not so loosely arranged as in *Sample 1*. The length of cells below the leaf epidermis is 2–3 times their width, while cells towards the middle of the mesophyll are shorter, at most twice as long as they are wide.

The spongy parenchyma consists of 3–4 rows of roundish, oval or irregularly shaped cells; here and there some longish cells similar to those in the palisade parenchyma are found; the latter are at most twice as long as they are wide. These cells, like those in the palisade parenchyma, are fairly closely set. The thickness of the leaf blade is not uniform (Fig. 5B); there is a moderate number of protrusions and depressions on the upper surface and considerably more on the lower leaf epidermis. On the lower surface of the leaf the veins protrude sharply. The vascular bundles occupy approximately half the thickness of the leaf and are found close together, in places at a distance of only 17–23 μm . The leaf blade is thinner (Fig. 1) than in the control and in *Sample 2* of the 100 ppm treatment, but in comparison to *Sample 1* marked thickening can be observed. The length of the palisade parenchyma cells (Fig. 2) has notably decreased compared to the control, to *Sample 2* of the 100 ppm treatment and to *Sample 1* alike. The cells of the spongy parenchyma (Fig. 2) have become substantially shorter than in the control, but only slightly so in comparison to *Sample 2* of the 100 ppm treatment; however, when compared to *Sample 1* they show some increase in length.

Cell-walls in the upper leaf epidermis (Fig. 3) are much thinner than in the control. When compared to *Sample 2* of the 100 ppm treatment a slight thickening is found, while in comparison with *Sample 1* a slight thinning can be observed. On the abaxial surface of the leaf the walls of the epidermis cells are very thin compared to the control (Fig. 3); the extent of thinning is minimal in comparison to *Sample 2* of the 100 ppm treatment and to *Sample 1*.

Sample 3 (11th July). Cells in the palisade parenchyma (Fig. 7C) are arranged in 1–3 rows in a similar way to those of *Sample 2*. The length of the outer cells is 2–3 times their width, while the cells in the inner row are at most twice as long as they are wide.

The 1–3 cell-rows of the spongy parenchyma (Fig. 7I) resemble the palisade parenchyma as regards the arrangement of the cells. The cells are generally longish, occasionally roundish, oval or of irregular shape. The longish cells are twice as long as they are wide.

The leaf blade is not of uniform thickness (Fig. 5C). Although larger or smaller protrusions and depressions are found on the upper leaf epidermis, those on the epidermis of the lower surface of the leaf are considerably larger. Laminar and angular collenchyma layers, located like islands below the epidermis are found on both the upper and lower sides of the leaves. On the lower leaf surface the veins protrude to a considerable extent. The vascular bundles are closely packed, at some places at a distance of only 23–29 μm , and occupy one-third (at the leaf edges more than half) the thickness of the leaf blade.

The thickness of the leaf blade shows a considerable increase compared both to the control, to *Sample 3* of the 100 ppm treatment and to *Sample 2* (Fig. 1).

The length of the palisade parenchyma cells (Fig. 2) has been markedly reduced compared to the control, to *Sample 3* of the 100 ppm treatment and to *Sample 2*.

The cells of the spongy parenchyma (Fig. 2) are somewhat shorter than in the control, but slightly longer than in *Sample 3* of the 100 ppm treatment and in *Sample 2* of the 1000 ppm treatment.

The difference in cell length between the palisade and spongy parenchyma has decreased to a minimum.

The thickness of the cell-walls in the upper and lower leaf epidermis has further decreased compared to the control, to *Sample 3* of the 100 ppm treatment and to *Sample 2* of the 1000 ppm treatment (Fig. 3).

Effect of 5000 ppm dicamba

Sample 1 (14th June). The cells of the palisade parenchyma (Fig. 7D) are arranged in 1–2 rows and are not closely packed. The length of the cells below the epidermis is 3–4 times their width, while cells towards the middle of the mesophyll are 2–3 times longer than they are wide.

The spongy parenchyma (Fig. 7K) consists of 3–5 rows of loosely arranged cells. The cells are roundish, oval or occasionally of irregular shape.

The leaf blade is of uniform thickness (Fig. 5D); there are no protrusions or depressions on the upper surface and only a few on the lower leaf epidermis. The leaf veins do not protrude to any great extent.

The average distance between the vascular bundles is $36\text{ }\mu\text{m}$ ($34\text{--}38\text{ }\mu\text{m}$); they occupy some one-third of the thickness of the leaf blade. The leaf blade is only slightly thicker than that of the control, but considerably thicker compared to *Sample 1* of the 1000 ppm treatment (Fig. 1).

The cells of the palisade and spongy parenchyma are much shorter than in the control (Fig. 2); in comparison to *Sample 1* of the 1000 ppm treatment the difference in the length of the palisade parenchyma cells is slight, but the cells of the spongy parenchyma are somewhat elongated.

Cell-walls in both the upper and lower leaf epidermis are considerably thinner than in the control (Fig. 3), and even in comparison with *Sample 1* of the 1000 ppm treatment a slight degree of thinning can be observed.

Sample 2 (21st June). The palisade parenchyma (Fig. 7E) forms a layer 1–2 cell-rows thick with fairly close-set cells. Cells below the epidermis are 2–3 times longer than they are wide, while the length of those towards the middle of the mesophyll is at most twice their width.

The spongy parenchyma (Fig. 7L) consists of 3–4 rows of roundish, oval or irregularly shaped cells. The cells are spaced fairly close together, in a similar way to those in the palisade parenchyma.

The thickness of the leaf blade is relatively uniform (Fig. 5E); protrusions and depressions are found only on the lower leaf epidermis, and even these are of minor extent. On the lower surface of the leaf the veins protrude markedly.

The average distance between the vascular bundles is $34\text{ }\mu\text{m}$ ($32\text{--}36\text{ }\mu\text{m}$) and they occupy not quite one-third of the thickness of the leaf. The leaf blade is considerably thinner (Fig. 1) than in the control, in *Sample 2* of the 1000 ppm treatment or in *Sample 1* of the 5000 ppm treatment.

The cells of the palisade parenchyma are substantially shorter than in the control (Fig. 2); in comparison with *Sample 2* of the 1000 ppm treatment and *Sample 1* of the 5000 ppm treatment the difference is less.

The spongy parenchyma cells (Fig. 2) are much shorter than in the control, but differ only very slightly from the corresponding cells in *Sample 2* of the 1000 ppm treatment and *Sample 1* of the 5000 ppm treatment.

The walls of epidermis cells on the adaxial surface of the leaf (Fig. 3) are considerably thinner than in the control; in comparison with *Sample 2* of the 1000 ppm treatment and *Sample 1* of the 5000 ppm treatment, on the other hand, the extent of thinning is only slight.

The walls of epidermis cells (Fig. 3) on the abaxial surface of the leaf are substantially thinner than in the control; in comparison to *Sample 2* of the 1000 ppm treatment a slight thinning can be observed, while there is no difference in the thickness of the cell-walls compared to *Sample 1* of the 5000 ppm treatment.

Sample 3 (11th July). The palisade parenchyma consists of 1–2 rows of fairly close-spaced cells (Fig. 7F). Cells immediately below the epidermis are 3–4 times longer, and those towards the middle of the mesophyll 1–2 times longer than they are wide.

The cells of the spongy parenchyma (Fig. 7M) are arranged in 3–4 rows; they are roundish, oval or of irregular shape. The shape of some cells resembles that of the palisade parenchyma cells; these are twice as long as they are wide. The cells are fairly close-set, in much the same arrangement as in the palisade parenchyma.

The thickness of the leaf blade is relatively uniform (Fig. 5F); minor protrusions and depressions are found only on the lower leaf epidermis. The leaf veins on the abaxial surface protrude to a considerable extent. The vascular bundles are closer to one another and are occasionally found side by side. The average distance between them is 28 μm (24–32 μm); they occupy one-third of the thickness of the leaf blade. In comparison to the control and the previous sample the leaf blade shows some thickening (Fig. 1), but it is thinner than in Sample 3 of the 1000 ppm treatment.

The cells of the palisade parenchyma (Fig. 2) are substantially shorter than in the control; in comparison with the previous sample they show a slight increase in length, and a considerable increase compared to Sample 3 of the 1000 ppm treatment.

The cells of the spongy parenchyma are hardly any shorter than the corresponding cells of the control. They show a slight increase in length compared to the previous sample, and some reduction in comparison with Sample 3 of the 1000 ppm treatment.

The cell-walls in the upper leaf epidermis (Fig. 3) are thinner than in any other sample; the thinning of the cell-walls in the lower epidermis is very great compared to the control, while there is no difference in comparison with the previous samples in the 5000 ppm treatment (Fig. 3).

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PROLINE STAINING AS A NEW METHOD FOR DETERMINING THE VITALITY OF POLLEN GRAINS IN WIND AND INSECT POLLINATED PLANTS

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In five plant species of the family Leguminosae, Compositae and Gramineae, respectively, the proline content of the pollen was found to be in direct ratio to its vitality.

We have elaborated a new isatin staining method by which the pollen grains assume quite different colours according to their respective proline concentrations. The colours obtained through a differentiated staining of pollen grains by our isatin reagent indicate the different degrees of vitality.

The results of proline staining were supported by the percentage values of in vitro pollen germination.

As a considerable advantage of the new staining method in comparison to other techniques, the former vitality levels of fixed pollen grains can also be approximately determined with it, if within 24 hours following the pollination the pollen grains are dried at 90 °C and stored in dark, hermetically sealed until the examination.

The isatin staining of the pollen grains can be used in the case of insect- and wind pollinated species alike, if the proline content of the pollen grains exceeds 1.0 per cent of the drymatter, that is, if the species has "proline type" pollen.

Introduction

It has been found that the proline content of amino acid extracts from the pollens of many plant species is very high and is in direct ratio to its vitality (LINSKENS and SCHRAUWEN 1969, DASHEK and HARWOOD 1974, BRITIKOV 1975, YAMADA and KONO 1976, AHOKAS 1978, ZHANG et al. 1982, etc.).

It has also been reported that when herbaceous mesophytic plant species are grown in dry, cold or salty soil the proline in their leaves accumulates in an extremely great measure, its concentration even amounting to 2-3 per cent of the dry-matter (KUDREV 1970, ASPINALL et al. 1973, BATES et al. 1973, TYMMS and GAFF 1979, LEWITT 1980, PÁLFI et al. 1978, PÁLFI 1980, TYANKOVA et al. 1982).

It is known that the optimum water content in the leaves of herbaceous mesophytic plant species is usually 80-90 per cent of the fresh weight. At the same time the water content of mature pollen is maximum 40-50 per cent of the fresh weight, or even less. The pollen can therefore be regarded as cells with extremely reduced water content. That is supposed to be one of the reasons why in the pollens of many plant species the proline reaches 2 per cent of the dry-matter, as much as in the leaves of highly water deficient plants (PÁLFI et al. 1981).

ZHANG and CROES (1983) germinated pollens of *Lilium longiflorum* L. in vitro. In the germination medium of some variants they dissolved ¹⁴C-proline isotope, while the culture medium of the controls did not contain exogenous proline. The control and a part of the proline treatments were germinated at too high and too low temperatures, respectively,

while other variants in water deficient media. According to their results the pollens having taken up exogenous proline germinated substantially better at both the optimum and the extreme temperatures, and even in the water deficient media. The authors assume that in the course of the germination of pollens of numerous plant species the high proline concentration enhances the resistance in the case of unfavourable temperature and water conditions, increasing thereby the chances of successful fertilization. Similar conclusion was arrived at by KURSANOV and RUZHKOV (1980) who germinated pollens of *Ribes nigrum* L. in vitro and found that in media containing a larger quantity of proline the length of the tube grew 2–3-fold.

It has been pointed out that in the course of pollen germination the proline plays an important role as activator of the citrate cycle, source of nitrogen; further, it controls the water regime, maintains the function of enzymes, and in the form of hydroxyproline is an important building element of proteins in the wall of the pollen and the tube (TUPY 1963, LINSKENS 1974, BRITIKOV 1975, AHOKAS 1978, HESLOP-HARRISON 1979, DASHEK and MILLS 1981, ZHANG et al. 1982).

Our present work has the aim of making up a reagent which stains the pollen grains different colours according to their proline content, whereby we may obtain information about the degree of vitality.

Isatin is known to give an extremely good reaction with proline and forms with it an intensive blue or dark blue colour, if the development is carried out at 90 °C. At the same time, with amino acids other than proline the isatin gives a very poor reaction appearing in a pale yellowish or brownish colour. If the pollen grain contains a considerable amount of proline, the latter may form with the isatine a dark blue colour on the wall of the pollen (exine), which suppresses (stains over) the pale colours of the other amino acids.

Further, we examined whether the suitable isatin reagent could be equally used for live- and fixed pollen grains, as well as for wind- and insect pollinated species, respectively.

The results of our new pollen staining method are compared with the in vitro germination percentages of pollen grains and with the proline contents of amino acid extracts from pollen grains as well.

Material and method

In the family Leguminosae pollens from the insect pollinated species *Trifolium hybridum* L. and *Lotus corniculatus* L. were examined. Inflorescences were cut off together with the shoots, and the anthers were prepared in laboratory.

From *Helianthus annuus* L. mixed pollen was collected from different parts of the capitulum. In the case of *Chrysanthemum hortorum* L. pollen was obtained first from the outer circle of the capitulum, then a day later from an inner ring towards the centre, and next day from the centre of the capitulum.

Designations for the wind pollinated varieties of *Secale cereale* L., family Gramineae, and for the inbred lines of *Zea mays* L. are given in Table 1. The rye- and maize pollens were collected on the day of the greatest scatter.

The proline contents of amino acid extracts from the pollen were measured according to ASPINALL et al. (1973).

For the purpose of pollen staining we tried out solutions and concentrations of the isatin reagent prepared with several hundred kinds of solvent. It had to be taken into consideration that the solubility of proline in water and certain organic acids was far the highest of all amino acids (at 25 °C 166 g proline is dissolved in 100 ml distilled water!). Therefore such a basic solvent of the isatin was required — e.g. acetone — that would not dissolve either the proline or other amino acids. At the same time, the isatin reagent had to contain a minimum amount of organic acid (or an aqueous solution of it) to dissolve the free proline from the cell by penetrating the pollen grain to be examined, enabling thereby the isatin reaction to take place on the exine. If the reagent contains but slightly more than necessary

Table 1

Comparison of *in vitro* germination percentages of pollen grains
to the proline contents of pollen extracts and to the results of isatin staining
(vitality staining)

| Plant families, species and varieties | In vitro germination | Proline content (in dry matter) % | Positive staining with isatin |
|--|-------------------------|---|-------------------------------------|
| <i>Leguminosae</i> | | | |
| <i>Trifolium hybridum</i> * | 71 | 1.64 | 76 |
| <i>Lotus corniculatus</i> * | 68 | 1.56 | 74 |
| <i>Compositae</i> | | | |
| <i>Helianthus annuus</i> * | 72 | 0.03 | — |
| <i>Chrysanthemum hortorum</i> *, flowers of the outermost circle of the capitulum | 81 | 1.84 | 94 |
| <i>Ch. hortorum</i> *, flowers of the second circle of the capitulum | 66 | 1.50 | 73 |
| <i>Ch. hortorum</i> *, flowers of the centre of the capitulum | 45 | 1.31 | 47 |
| <i>Gramineae</i> | | | |
| <i>Secale cereale</i> **, cultivar S344 | 83 | 2.02 | 94 |
| <i>Secale cereale</i> **, cultivar S361 | 56 | 1.73 | 63 |
| <i>Secale cereale</i> **, cultivar S382 | 45 | 1.26 | 49 |
| <i>Zea mays</i> **, inbred line Z326 | 87 | 2.54 | 97 |
| <i>Zea mays</i> **, inbred line Z340 | 68 | 1.82 | 73 |
| <i>Zea mays</i> **, inbred line Z366 | 44 | 1.37 | 46 |

Average deviation below ± 5 per cent; $n = 3$.

* insect pollinated

** wind pollinated

of the solvent of proline, then the isatin reaction stains not only the exine but dissolves into the medium and stains it an intensive blue colour, whereby false results are obtained. At the same time, isatin must be in excess in the medium indicated by a crystallization after the staining.

For the new isatin reagent first 0.01 M citric acid was prepared, that is, 0.21 g citric acid was dissolved in 100 ml distilled water. The composition of the reagent: to 100 ml acetone 1.5 ml of the 0.01 M citric acid is added and 0.5 g isatin dissolved in it. Considering that this isatin reagent can be stored in refrigerator for 2–3 weeks without changing, only half or one-fifth of the given amount should be prepared at a time, and when it is used up, the reagent is newly made.

Staining was carried out on the slide of the microscope. 1–2, or 5–10 mg of pollen were placed with a lancet in the middle of the slide, and a drop of isatin reagent added with an eye-dropper to it. The pollen grains were immediately thoroughly mixed with the solution with the aid of the lancet, until the acetone completely evaporated. Staining with a drop of isatin reagent, and mixing were repeated twice. Then the slide with the pollen was heated for 10 minutes in an exsiccator of 90 °C, to develop the colours. When the slide removed from the exsiccator cooled down the yellow excess of stain round the pollen was wiped off with wet cotton by means of a forceps. Subsequently on the mass of pollen stained dark and stuck on the slide a tiny drop of paraffine oil was applied with a glass-rod, the pollen grains thoroughly mixed (dispergated) in it, and gathered in the middle of the slide, and finally covered with a slide and slightly pressed.

When alive pollen grains are stained, the acetone in the reagent, and the development of colour at 90 °C fix them. Alive pollen has to be fixed at 90 °C and dried on the day of collecting. In that case with pollen samples stored in small air- and light-tight flasks determination by staining can be carried out even 5–6 months later.

The colours of staining with the isatin reagent were examined by microscope as magnified 100–300 times. With five fields of vision surveyed even an approximate percentage evaluation could be carried out.

For the purpose of *in vitro* germination the solid culture medium was prepared on the basis of a publication by STANLEY and LINSKENS (1974). Each examination and analysis was carried out in 3 replications, and the average results were recorded. If the deviation of even a single result of an analysis from the average exceeded $\pm 5\%$, the whole analysis was repeated.

Results and discussion

In the course of the microscope examination of pollen grains stained with the isatin reagent it was found that in pollen grains with high free proline concentrations the cell-walls were stained dark blue or black in consequence of a reaction with the isatin. If the proline content was lower the walls of the pollen grains assumed a blue, light blue or greenish blue colour. And when the pollen grains contained proline in quite small quantities or in traces, their walls turned brownish yellow, or were not stained at all, that is, retained their original pale yellow colour.

On the black-and-white photo the pollen grains stained dark blue or black appear equally in black (Fig. 1). The other colours give various shades of grey. In Fig. 1 the quite light grey pollen grains are sterile.

From Fig. 1 it can thus be established that the pollen grains do not contain equal amounts of proline, and the new method of isatin staining indicates the proline contents and the degrees of vitality by a sharp differentiation of colour.

In the insect pollinated species *Chrysanthemum hortorum* L. pollen grains collected from various parts of the capitulum were found to show quite different degrees of vitality.

We also pointed out that our new method of staining gave identical results with the alive and fixed pollen grains of the same sample.

The data of Fig. 1 give evidence of a wide variation of vitality of the pollen of different rye varieties and inbred maize lines.

In the course of examining pollen samples collected from the same varieties of rye and maize in 3 successive years we found that the vitality degrees of pure varieties or inbred maize lines remained relatively unchanged from year to year, that is in the stable cultivated species the vitality level of the pollen can be regarded as a specific character if the plants are grown under optimum conditions.

It should be noted that the degrees of vitality of pollen grains in the different rye- and maize varieties generally are not as extreme as shown by Fig. 1. In most varieties the vitality of pollen is about 70 per cent. For the sake of demonstration we deliberately looked for varieties with a very high vitality of pollen grains, as well as one with a large number of sterile pollen grains.

As seen from Table 1 the new isatin reagent can be used for determining the vitality level of pollen in the examined species of the families Leguminosae, Compositae and Gramineae except *Helianthus annuus* which while showing a 72 per cent in vitro germination contained free proline in the amino acid extracts at a minimum concentration.

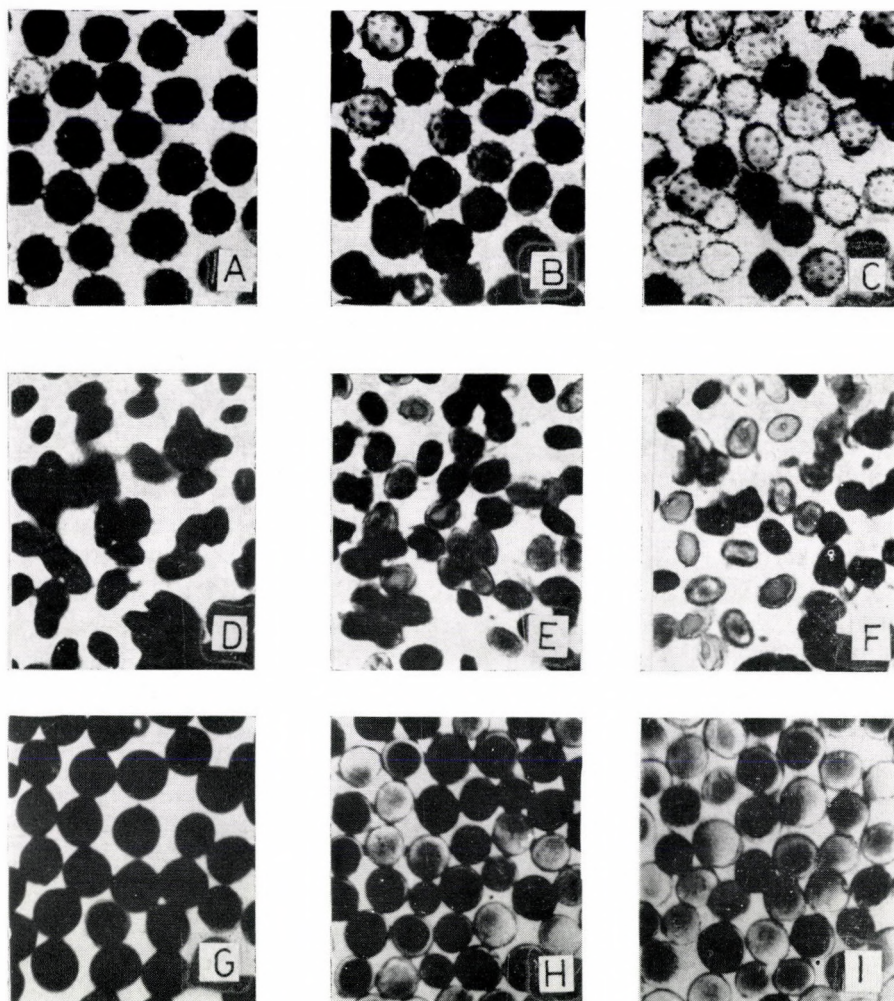


Fig. 1. The proline contents of pollen grains in the species *Chrysanthemum hortorum* (ABC) *Secale cereale* (DEF) and *Zea mays* (GHI) are in direct ratio with their degrees of vitality. Our isatin reagent stains the viable pollen grains dark blue or black. Pollen grains of low vitality become light blue or greenish blue (in the figure various shades of grey). Sterile pollen grains do not stain, they remain yellow (in the picture light grey). A = pollen obtained from the outermost circle of the capitulum of *Chrysanthemum hortorum*; B = pollen grains from the second circle of the capitulum; C = pollen grains collected from the centre of the capitulum. DEF = pollen grains from three cultivars of *Secale cereale* (S344, S361, S382). GHI = pollen grains from 3 inbred lines of *Zea mays* (Z326, Z340, Z366)

Table 1 reveals that on the basis of the proline contents of pollen grains vitality can be determined by the new method of staining both in insect and wind pollinated species.

It also can be seen that staining with the isatin reagent gave in each case a somewhat higher percentage of vitality than the percentage of in vitro germination. It can be supposed, however, that apart from the proline content other factors inhibiting the germination may also occur in the pollen grains.

According to Table 1 the highest value of vitality percentage pointed out by staining was obtained with the pollen sample taken from the outermost circle of the capitulum of *Chrysanthemum hortorum*, as also shown by Fig. 1. Towards the centre of the capitulum the vitality of the pollen considerably decreases. This statement is supported by the data of germination and the proline contents of the pollen extracts.

In the *Secale cereale* varieties and inbred *Zea mays* lines in Table 1 the vitality percentage pointed out by isatin staining is shown to change with the same tendency as the in vitro germination. The proline contents of amino acid extracts of pollen display the same tendency of change.

From the results of the table we can draw the conclusion that the isatin reagent can be used only for species with a proline concentration exceeding 1.0 per cent of the pollen dry weight. In *Helianthus annuus* the proline content is much lower (0.03 per cent). The pollen of species like that may be called "non-proline type" pollen.

Higher than 1.0 per cent proline content of viable pollen was pointed out by TUPÝ (1963) in apple, by LINSKENS and SCHRAUWEN (1969) in *Petunia hybrida*, by STANLEY and LINSKENS (1974) in *Cynodon dactylon*, by YAMADA and KONO (1976) in rice, by AHOKAS (1978) in barley and by KURSAKOV and RYZHKOV (1980) in *Ribes nigrum*. The authors unequivocally state that the high proline content of pollen in the above plant species, though not the cause, is undoubtedly an indicator of vitality.

The vitality of pollen grains can be determined in many ways: e.g. by in vitro germination, or colouring matters like the aniline blue, the TTC, the acetocarmine, etc. However, the above listed and all other methods applied so far only point out how many of the pollen grains were live or dead at the beginning of the examination. Our new method has the very advantage that when the mature pollen grains are fixed on the day of collecting their vitality can be determined even weeks later, that is, the method does not require live pollen.

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RESULTS OF PHYTOPHENOLOGICAL OBSERVATIONS IN THE POPLAR CLONE-TESTING AREA (POPULETUM) NEAR KECSKEMÉT

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Four phenophases of 64 different poplar clones were observed during 4 subsequent years. Their bursting time, "green peak" period, growth period and foliation were related to temperature and rainfall data and coincidences were studied.

Introduction

On the initiative of the Országos Erdészeti Főigazgatóság (OEF) (National Chief Board of Woods and Forests) poplar clone-testing areas were established in eight forestries at the end of the 1950s.

The clone-collections were introduced with the intention of improvement. It was a desire to make a comparison between the best clones of our native poplars in the populetum region by region and niche by niche as well as to compare the clones proved good abroad with the clones improved from our best plus-trees and the euramerican poplars grown in our country up to now.

It was a purpose to select and propagate clones which are best suitable for the ecological conditions of the Hungarian sites and resist diseases.

Such a poplar plantation (Populetum) can be found near Kecskemét, central Hungary, too. The 64 species and clones, respectively, introduced in 1961 gave a favourable opportunity for observations of phenology and growth dynamics.

General description of the populetum

The populetum was established in a spacing of 10×10 m in the spring of 1961. The soil is sandy meadow-soil and its combination with other soil types, respectively. It belongs to the hydrological category where the effect of ground-water is periodical or permanent. Its climate is that corresponding to forest-steppe vegetation. Due to the spacing of plantation of 10×10 m it seemed to be suitable for carrying out phenological observations as practically no oppressed individuals could be found. Each tree individual could tolerate the weather effects to the same extent.

Methods

The phenological observations were being carried out the *Populetum* through four years (from 1976 to 1979). During our observations the times of the following phenophases were recorded clone by clone:

Table 1
Climatological data

| Year | Monthly average temperature in °C | | | | | | | | | | | | Annual average, °C | Average of growing period 1. IV.–30. IX. |
|------|-----------------------------------|------|------|------|------|------|------|-------|------|------|-----|------|--------------------|--|
| | I. | II. | III. | IV. | V. | VI. | VII. | VIII. | IX. | X. | XI. | XII. | | |
| 1976 | 0.0 | −0.7 | 2.3 | 12.2 | 16.4 | 19.5 | 22.5 | 18.4 | 15.2 | 11.9 | 7.0 | 0.2 | 10.4 | 17.4 |
| 1977 | 0.3 | 4.5 | 8.9 | 9.6 | 17.1 | 20.7 | 20.6 | 19.8 | 14.1 | 12.1 | 5.1 | −1.9 | 10.9 | 17.0 |
| 1978 | 0.0 | 0.4 | 7.0 | 10.3 | 14.0 | 18.6 | 19.7 | 18.7 | 14.6 | 11.1 | 1.5 | 1.2 | 9.8 | 16.0 |
| 1979 | 2.5 | 1.9 | 8.0 | 10.2 | 18.2 | 22.5 | 19.2 | 20.1 | 17.5 | 9.6 | 5.3 | 3.9 | 11.6 | 17.95 |

| Year | Monthly rainfall in mm | | | | | | | | | | | | Total | During the growing period 1. IV.–30. IX. |
|------|------------------------|------|------|------|------|------|------|-------|-------|------|------|-------|-------|--|
| | I. | II. | III. | IV. | V. | VI. | VII. | VIII. | IX. | X. | XI. | XII. | | |
| 1976 | 46.8 | 2.1 | 40.3 | 43.4 | 33.6 | 45.4 | 31.7 | 23.2 | 112.7 | 54.5 | 29.7 | 103.2 | 526.6 | 290.0 |
| 1977 | 26.1 | 74.9 | 50.1 | 22.2 | 25.5 | 58.1 | 64.6 | 31.5 | 39.2 | 13.8 | 79.8 | 25.4 | 511.2 | 241.1 |
| 1978 | 8.9 | 34.1 | 30.4 | 37.7 | 65.1 | 95.0 | 63.6 | 35.3 | 69.9 | 9.5 | 5.9 | 53.9 | 509.3 | 366.6 |
| 1979 | 57.6 | 30.6 | 36.3 | 37.3 | 14.0 | 67.5 | 69.3 | 36.6 | 11.3 | 18.9 | 69.1 | 59.8 | 508.3 | 236.0 |

| Year | Number of sunny hours every month | | | | | | | | | | | | Total | During the growing period 1. IV.–30. IX. |
|------|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|------|--------|--|
| | I. | II. | III. | IV. | V. | VI. | VII. | VIII. | IX. | X. | XI. | XII. | | |
| 1976 | 41.9 | 100.3 | 130.4 | 169.5 | 225.2 | 264.6 | 242.1 | 189.0 | 103.3 | 100.7 | 58.8 | 37.4 | 1663.2 | 1193.7 |
| 1977 | 47.5 | 86.0 | 153.8 | 150.6 | 189.6 | 207.3 | 216.8 | 191.9 | 170.3 | 143.0 | 70.9 | 37.9 | 1665.6 | 1126.5 |
| 1978 | 66.2 | 50.3 | 140.2 | 156.7 | 150.0 | 201.9 | 230.4 | 219.1 | 148.9 | 156.2 | 10.4 | 27.3 | 1557.6 | 1107.0 |
| 1979 | 45.1 | 68.6 | 152.2 | 194.0 | 241.3 | 215.3 | 204.7 | 209.7 | 187.0 | 152.9 | 27.7 | 48.1 | 1746.6 | 1252.0 |

Table 2
Phenologic analysis of Leuce poplar clones

| Clones made by selection or introduction | | Origin |
|--|----------|-----------------------------------|
| Serial number | Clone | |
| 2. <i>P. tremula</i> | | Sátoraljaújhely 184. plus-tree |
| 9. <i>P. × canescens</i> Sm | | Ráckeve 143. plus-tree |
| 10. <i>P. × canescens</i> Sm | | Nagyrezét 52. plus-tree |
| 32. <i>P. alba</i> | | Kunpeszér 175-1 plus-tree |
| 33. <i>P. alba</i> | | Kunpeszér 175-2 plus-tree |
| 35. <i>P. alba</i> | | Poland |
| Crossed clones | | |
| 18. <i>P. canescens</i> × cv. 'bolleana' | H 372-1 | Bugac 132 × Lajosmizse 176. |
| 19. <i>P. canescens</i> × cv. 'bolleana' | H 372-2 | Bugac 132 × Lajosmizse 176. |
| 20. <i>P. canescens</i> × cv. 'bolleana' | H 372-4 | Bugac 132 × Lajosmizse 176. |
| 24. <i>P. canescens</i> × cv. 'bolleana' | H 428-3 | Nagyrezét 52 × Lajosmizse 176. |
| 31. <i>P. canescens</i> × <i>P. alba</i> | H 441-8 | Nagyrezét 52 × Kunpeszér 181. |
| 36. <i>P. alba</i> × <i>P. tremuloides</i> | H 456-1 | Kunpeszér 175 × Canada Angus 3. |
| 37. <i>P. alba</i> × <i>P. tremuloides</i> | H 456-2 | Kunpeszér 175 × Canada Angus 3. |
| 38. <i>P. alba</i> × <i>P. tremuloides</i> | | Czechoslovakia Spalek crossing |
| 39. <i>P. alba</i> × <i>P. grandidentata</i> | H 422-1 | Alsónémedi 140. × Canada, Ontario |
| 40. <i>P. alba</i> × <i>P. grandidentata</i> | H 422-6 | Alsónémedi 140. × Canada, Ontario |
| 43. <i>P. alba</i> × <i>P. alba</i> | H 425-4 | Alsónémedi 140. × Kunpeszér 181. |
| 44. <i>P. alba</i> × <i>P. alba</i> | H 425-10 | Alsónémedi 140. × Kunpeszér 181. |

- beginning and end of flowering
- beginning of budding — complete foliation
- beginning of colouration of leaves — complete discoloration of leaves
- beginning of defoliation — complete defoliation

The data of the daily average temperature and rainfall of the forestry meteorological station of Kerekegyháza lying approximately 6 kms away from the Populetum were also collected (Table 1).

The total series of data covering the 64 clones can be found in the archives of the Danube-Tisza Midregion Experiment Station of the Forest Research Institute (Erdészeti Tudományos Intézet Duna-Tisza közti Kísérleti Állomása).

Further on, 18 clones belonging to the Leuce section being most important with a view to forestry and 10 clones belonging to the Aigeiros section will be analyzed in detail (Table 2).

Later on, the clones will be described on the basis of their serial number because they can be separated mostly in this way.

Flowering

With poplars, the beginning of the growing period is marked by the beginning of the flowering, appearing of the catkins. The last frosty day in 1976 was on 22nd March, the 83rd day of the year and up to that time the daily average temperature rose not once over +8 °C. On March 30th, 31st and in the first week of April the daily mean temperature was above 10 °C. Flowering of the poplars began almost at the same time, from March 31st to April 2nd, accordingly rather late. In the last week of February in 1977 the daily mean temperature was 8 to 12 °C through several days not regarding the fact that there were four cold days (0 to +5 °C) early in March and catkins appeared on March 3rd. Under the influence of the

cold weather flowering did not begin at the same time: the clone No. 36 (*P. alba* × *P. tremuloides* H 456-1) began to bloom first, on March 4th while the clone No. 43 (*P. alba* × *P. alba* H 425-4) last, on March 16th.

In the first week of March in 1978 the daily average temperatures alternated between 9 and 12 °C. Again the clone No. 36 burst into bloom earliest on March 7th while the clone No. 18 (*P. canescens* × cv. '*bolleana*' H 372-1) at latest, on March 30th.

A very interesting thing happened in 1979. Flowering (9. *P. × canescens* Sm.) began on February 24th although the daily average temperature was -1.1 °C and it didn't rise above 4 °C in the preceding week. The other Leuce poplars, too, flowered on the first 10 days of March although the daily average temperature was between 3 and 8 °C.

Of the studied four years flowering began three times early in March but in 1976 it began only at the end of March (three weeks later).

In 1976 the average temperature in January was 0 °C, in February -0.7 °C, in March 2.3 °C, consequently there was a cold winter and early spring.

In 1977 the average temperature in January was 0.3 °C, in February 4.5 °C, in March 8.9 °C, consequently the temperature conditions were considerably more favourable than those in the preceding year, this may be the reason of the earlier flowering. Considering the average of the last three years the beginning of the flowering was worked out as follows.

| Early-flowering poplars | Day of the beginning of flowering | Deviation in days |
|---|-----------------------------------|-------------------|
| 36. <i>P. alba</i> × <i>P. tremuloides</i> H 456-1 | 4. III. | ±3 |
| 2. <i>P. tremula</i> | 5. III. | ±4 |
| 9. <i>P. canescens</i> | 5. III. | +5 to 9 |
| Late-flowering poplars | | |
| 32. <i>P. alba</i> | 11. III. | ±5 |
| 31. <i>P. canescens</i> × <i>P. alba</i> H 441-8 | 14. III. | ±3 |
| 18. <i>P. canescens</i> × cv. ' <i>bolleana</i> ' H 372-1 | 14. III. | ±14 to 11 |
| 19. <i>P. canescens</i> × cv. ' <i>bolleana</i> ' H 372-2 | 15. III. | ±15 to 10 |

The other clones can be ranked amongst those flowering at average time.

Beginning of foliation

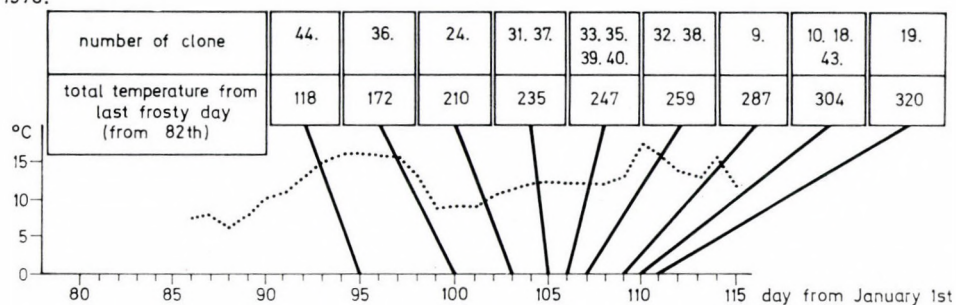
The phase of flowering is followed by the foliation, generally at the time when poplars have already dropped their catkins. It could be experienced during our observations that there can be a difference of 16 to 30 days between the beginning of the vegetation of the poplar clones flowering first and last. On the basis of the data obtained during the four years order of flowering of the 18 pcs of Leuce poplars is as follows.

In the Table 3 by total temperature the sum of the daily average temperature is to be meant, counted from the last frosty day.

It can be clearly seen in the Table 3 that every year the clone No. 44 (*P. alba* × *P. alba* H 425-10) is in bud earliest.

Drawing a parallel between the meteorological data and phenological observations it was established that if the first daily average temperature is above 10 °C becomes steady

1976.



1977.

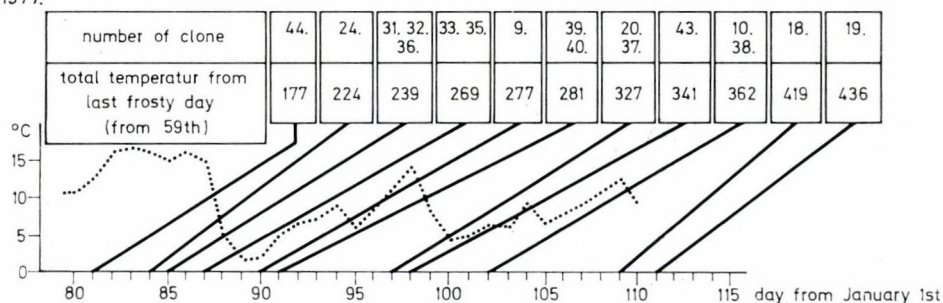
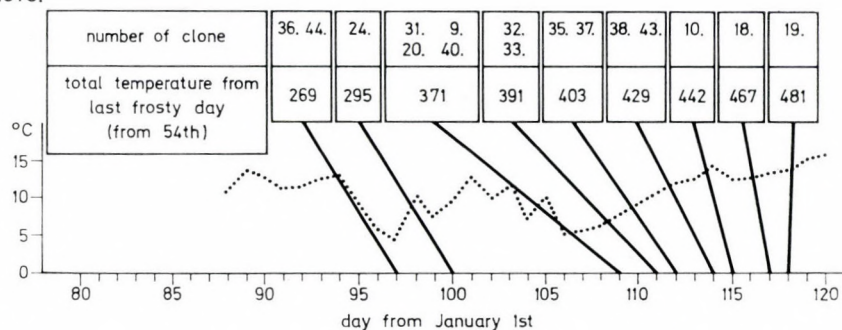


Fig. 1. Begin of bud burst of poplar varieties in the function of the heat-sum from the last frosty day, in the years 1976-77. — Daily average temperature in °C

1978.



1979.

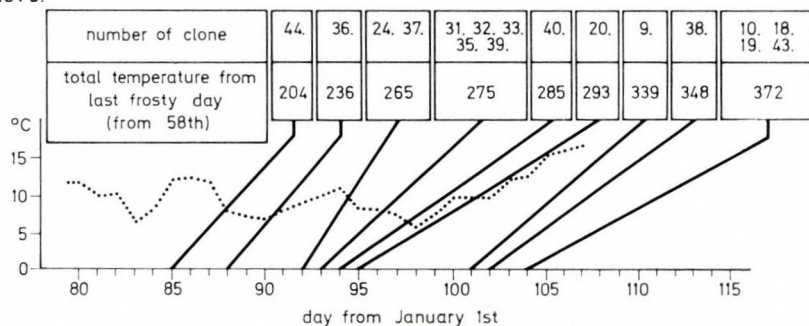


Fig. 2. Begin of bud-burst of poplar varieties in the function of the heat-sum from the last frosty day, in the years 1978-79. — Daily average temperature in °C

Table 3
Total temperature required for foliation

| Number of clones | Day of foliation from the beginning of the year | | | | | Total temperature, °C | Deviation day | |
|----------------------------------|---|------|------|------|----------|-----------------------------|---------------|----|
| | 1976 | 1977 | 1978 | 1979 | average | | + | — |
| Early-bursting ones | | | | | | | | |
| 44. | 95 | 81 | 97 | 85 | 31. III. | 192 | 7 | 9 |
| 36. | 100 | 85 | 97 | 88 | 3. IV. | 229 | 7 | 8 |
| 24. | 103 | 84 | 100 | 92 | 5. IV. | 249 | 8 | 11 |
| Poplars bursting at average time | | | | | | | | |
| 31. | 105 | 85 | 109 | 93 | 8. IV. | 280 | 11 | 13 |
| 32. | 107 | 85 | 111 | 93 | 9. IV. | 291 | 12 | 14 |
| 33. | 106 | 87 | 111 | 93 | 9. IV. | 296 | 12 | 12 |
| 39. | 106 | 91 | 111 | 93 | 10. IV. | 308 | 11 | 9 |
| 40. | 106 | 91 | 109 | 94 | 10. IV. | 296 | 9 | 9 |
| 35. | 106 | 87 | 112 | 93 | 10. IV. | 299 | 12 | 13 |
| 9. | 109 | 90 | 109 | 101 | 12. IV. | 319 | 7 | 12 |
| 20. | 107 | 97 | 109 | 95 | 12. IV. | 313 | 7 | 7 |
| 37. | 106 | 97 | 112 | 92 | 12. IV. | 313 | 10 | 10 |
| Late-bursting ones | | | | | | | | |
| 38. | 107 | 102 | 114 | 102 | 16. IV. | 350 | 8 | 4 |
| 43. | 110 | 98 | 114 | 104 | 16. IV. | 360 | 8 | 8 |
| 2. | 113 | 98 | 115 | 103 | 17. IV. | 372 | 8 | 9 |
| 10. | 110 | 102 | 115 | 104 | 18. IV. | 370 | 7 | 6 |
| 18. | 110 | 109 | 117 | 104 | 20. IV. | 391 | 7 | 6 |
| 19. | 111 | 111 | 118 | 104 | 21. IV. | 402 | 7 | 7 |

for 2 to 3 days early bursting clones (of Nos 44, 36, 24) begins. At the next rise in temperature the clones bursting at average time come into leaf then, at the third rise in temperature, those bursting late (Figs 1, 2).

Figures are showing the summarized total heat in °C, the daily average temperature as well as the day of bursting of the clones, from the last frosty day.

From the beginning of foliation to the complete foliation

The duration of this phenophase, too, is considerably influenced by the temperature. The higher is the mean of the daily average temperatures after budding the shorter time this phenophase will last.

It is shown in Table 4 how many days the total foliation takes from the beginning of budding and how much the average value of temperature was during this period.

In case of daily average temperature higher than 12 °C, the complete foliation generally lasts a period shorter than 10 days while in case of an average temperature about 7 to 8 °C it makes even three weeks.

Duration of the formation of the complete foliation depends not so much on the clone as on the beginning of budding.

Table 4
Phenological data of foliation

| Clone number | 1976 | | | 1977 | | | 1978 | | | 1979 | | |
|--|------------------------|---------------------------------|------------------------------------|------------------------|---------------------------------|------------------------------------|------------------------|---------------------------------|------------------------------------|------------------------|---------------------------------|------------------------------------|
| | Beginning of foliation | Time of complete foliation, day | Mean temperature of the period, °C | Beginning of foliation | Time of complete foliation, day | Mean temperature of the period, °C | Beginning of foliation | Time of complete foliation, day | Mean temperature of the period, °C | Beginning of foliation | Time of complete foliation, day | Mean temperature of the period, °C |
| Early-bursting ones | | | | | | | | | | | | |
| 44. | 5. IV. | 12 | 11.5 | 22. III. | 7 | 14.5 | 7. IV. | 17 | 9.5 | 26. III. | 19 | 8.9 |
| 36. | 10. IV. | 11 | 13.2 | 26. III. | 20 | 8.8 | 7. IV. | 21 | 10.1 | 29. III. | 16 | 8.6 |
| 24. | 13. IV. | 9 | 13.8 | 25. III. | 18 | 8.9 | 10. IV. | 16 | 9.9 | 2. IV. | 12 | 9.0 |
| The ones bursting at average time | | | | | | | | | | | | |
| 31. | 15. IV. | 7 | 14.2 | 26. III. | 17 | 8.5 | 19. IV. | 10 | 12.4 | 3. IV. | 15 | 9.9 |
| 32. | 17. IV. | 6 | 14.9 | 26. III. | 17 | 8.5 | 21. IV. | 11 | 13.8 | 3. IV. | 15 | 9.9 |
| 33. | 16. IV. | 6 | 14.6 | 28. III. | 23 | 7.3 | 21. IV. | 7 | 12.8 | 3. IV. | 15 | 9.9 |
| 39. | 16. IV. | 6 | 14.6 | 1. IV. | 21 | 7.3 | 21. IV. | 11 | 13.8 | 3. IV. | 18 | 9.5 |
| 40. | 16. IV. | 8 | 14.3 | 1. IV. | 19 | 7.0 | 19. IV. | 13 | 14.1 | 4. IV. | 15 | 9.6 |
| 35. | 16. IV. | 6 | 14.6 | 28. III. | 23 | 7.3 | 22. IV. | 10 | 14.4 | 3. IV. | 15 | 9.9 |
| 9. | 18. IV. | 5 | 13.8 | 31. III. | 24 | 7.3 | 19. IV. | 9 | 12.1 | 11. IV. | 9 | 10.7 |
| 20. | 16. IV. | 5 | 14.7 | 7. IV. | 20 | 8.8 | 19. IV. | 13 | 13.2 | 5. IV. | 13 | 10.0 |
| 37. | 15. IV. | 7 | 14.2 | 7. IV. | 15 | 8.9 | 22. IV. | 10 | 14.4 | 2. IV. | 15 | 9.9 |
| Late-bursting ones | | | | | | | | | | | | |
| 38. | 16. IV. | 5 | 14.7 | 12. IV. | 19 | 9.4 | 24. IV. | 8 | 14.2 | 12. IV. | 11 | 10.9 |
| 43. | 19. IV. | 6 | 16.0 | 8. IV. | 19 | 8.8 | 24. IV. | 8 | 14.2 | 14. IV. | 14 | 11.6 |
| 2. | 22. IV. | 12 | 10.8 | 8. IV. | 17 | 8.7 | 25. IV. | 8 | 14.2 | 13. IV. | 11 | 11.3 |
| 10. | 19. IV. | 18 | 12.7 | 12. IV. | 18 | 9.7 | 25. IV. | 11 | 14.7 | 14. IV. | 13 | 11.7 |
| 18. | 19. IV. | 10 | 10.7 | 9. IV. | 11 | 11.8 | 27. IV. | 6 | 14.6 | 14. IV. | 10 | 11.2 |
| 19. | 20. IV. | 12 | 10.5 | 11. IV. | 9 | 12.0 | 28. IV. | 8 | 15.3 | 14. IV. | 10 | 11.2 |

Duration of foliation and increase in tree volume

Length of time of foliation can be calculated

- a) from the beginning of budding to the beginning of discolouration of leaves,
- b) from the beginning of budding to the beginning of leaf-fall,
- c) from the beginning of budding to the complete defoliation.

The best is to calculate the duration of foliation from the beginning of budding to the beginning of defoliation because in this period there is a measurable increase in the tree volume, too. This period can also be called the period of "green peak".

According to the duration of foliation poplar clones of long, average, short and rather short "green peak" periods were separated (Table 5).

The hybrids *P. alba* × *P. alba*, *P. alba* × *P. tremula*, and *P. alba* × *P. grandidentata* are of long "green peak" period. The hybrids *P. canescens* and *P. canescens* × cv. 'bolleana' are clones of short and rather short growing periods.

With the exception of two clones, "green peak" period was longest in 1977. It can be explained by the fact that budding began earliest this year, due to the effect of the favourable average temperature in March of about 9 °C.

The order of the clones slightly changes if the duration of vegetation is calculated from the beginning of budding the complete defoliation in days.

Table 5
Length of "green peak" period in days

| Clone number | 1976 | 1977 | 1978 | 1979 | Average |
|---|------|------|------|------|---------|
| day | | | | | |
| Poplars of long "green peak" period | | | | | |
| 39. | 173 | 190 | 178 | 182 | 181 |
| 40. | 172 | 185 | 184 | 169 | 178 |
| 44. | 172 | 183 | 182 | 161 | 175 |
| 36. | 170 | 186 | 171 | 169 | 174 |
| Poplars of average "green peak" period | | | | | |
| 2. | 179 | 171 | 171 | 152 | 168 |
| 9. | 168 | 186 | 164 | 149 | 167 |
| 38. | 170 | 174 | 161 | 162 | 167 |
| 32. | 160 | 186 | 152 | 164 | 166 |
| 33. | 161 | 182 | 157 | 162 | 165 |
| 37. | 165 | 174 | 158 | 161 | 165 |
| 35. | 157 | 177 | 156 | 157 | 162 |
| 31. | 162 | 163 | 161 | 157 | 161 |
| Poplars of short "green peak" period | | | | | |
| 18. | 160 | 160 | 158 | 146 | 156 |
| 19. | 156 | 160 | 157 | 149 | 156 |
| 10. | 160 | 162 | 150 | 144 | 154 |
| Poplars of very short "green peak" period | | | | | |
| 43. | 153 | 161 | 135 | 132 | 145 |
| 20. | 146 | 155 | 135 | 141 | 144 |
| 24. | 160 | 150 | 144 | 123 | 144 |

It is rather interesting, however, that the length of "green peak" period of the clone No. 24 (*P. canescens* × cv. 'bolleana' H 428-3) from the beginning of budding to the beginning of defoliation is 144 days (it is rather short) and 70 days pass from the beginning of the defoliation to the complete defoliation. It is dropping its leaves during 1/3rd of the total growing period (in average 213 days) (Table 6).

Size of basal area increment of various clones under the influence of temperature and rainfall

In 1976 and 1977 the perimeter-growth of 12 poplars belonging to the Leuce section was measured weekly. In spring bronze-strips were placed on the stems in a height of 130 cm from the soil surface, according to the method of Z. JÁRÓ. Measuring the measuring points fixed onto the strip, the weekly increase in perimeter was obtained. The basal area increment was calculated in cm² from the growths of the anchor-ring, registered weekly. In Fig. 3 the rate of basal area growth of 1976 can be seen while Fig. 4 illustrates that of 1977 in the function of the weekly rainfall and average temperature.

Table 6

Length of the vegetation period of the studied poplars

| Clone number | 1976 | 1977 | 1978 | 1979 | Average |
|---------------------------------------|------|------|------|------|---------|
| day | | | | | |
| Clones of long vegetation period | | | | | |
| 44. | 212 | 231 | 222 | 214 | 220 |
| 39. | 208 | 231 | 208 | 226 | 218 |
| 40. | 210 | 229 | 210 | 222 | 218 |
| 36. | 212 | 221 | 217 | 210 | 215 |
| 35. | 208 | 229 | 202 | 218 | 214 |
| 24. | 206 | 224 | 214 | 208 | 213 |
| 32. | 207 | 227 | 208 | 209 | 213 |
| 31. | 209 | 219 | 207 | 209 | 211 |
| Clones of middle vegetation period | | | | | |
| 33. | 201 | 219 | 197 | 207 | 206 |
| 2. | 207 | 214 | 204 | 197 | 206 |
| 9. | 203 | 214 | 205 | 199 | 205 |
| 38. | 209 | 204 | 198 | 207 | 205 |
| Clones of short vegetation period | | | | | |
| 37. | 202 | 191 | 200 | 203 | 199 |
| 10. | 202 | 204 | 197 | 194 | 199 |
| 19. | 198 | 195 | 196 | 205 | 199 |
| 18. | 197 | 195 | 197 | 201 | 198 |
| 43. | 202 | 192 | 184 | 191 | 192 |
| Clone of very short vegetation period | | | | | |
| 20. | 195 | 189 | 189 | 180 | 188 |

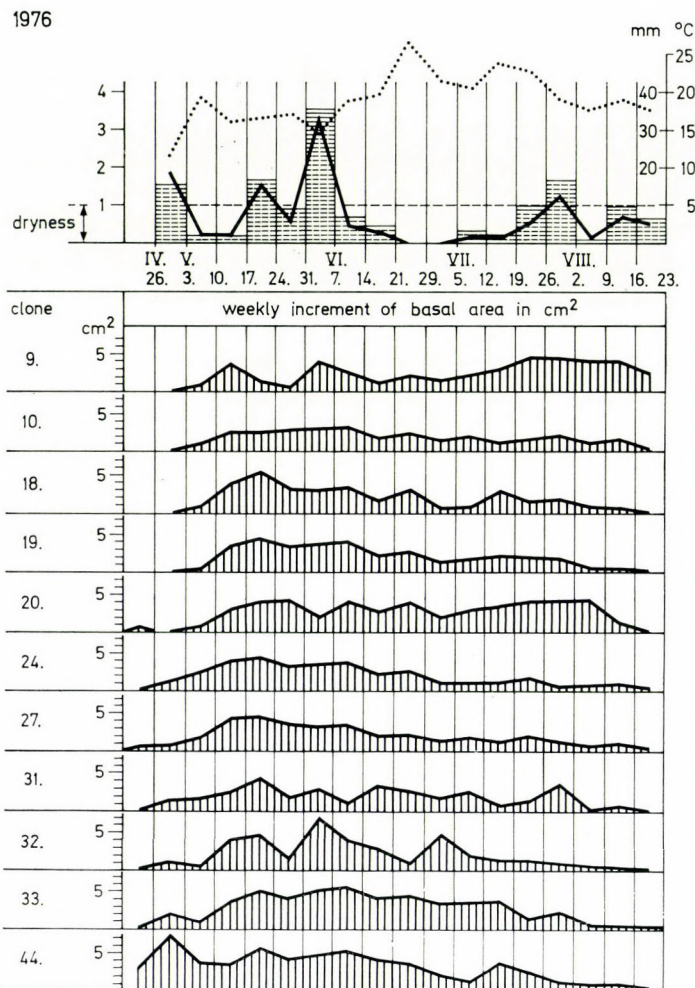


Fig. 3. Weekly basal area growth in cm^2 in the function of weekly temperatures and precipitation in 1976

To ensure the essential conditions of the plants also 1 mm optimal rainfall in every 10°C would be desirable in the growing period. Hence from the following formula if the period was dry or wet:

$$\frac{\text{Sum of weekly rainfall (mm)} \times 10}{\text{Sum of daily average temperatures}}$$

If this value is less than 1 we can speak of a dry period. Figures are showing clearly that there was a considerable basal area increment when the value calculated according to the formula was greater than 1 or was quite near to 1.

The increase in thickness of the Leuce poplars generally lasts by the end of August, thus it is expedient to count the "green peak" period to the beginning of the defoliation.

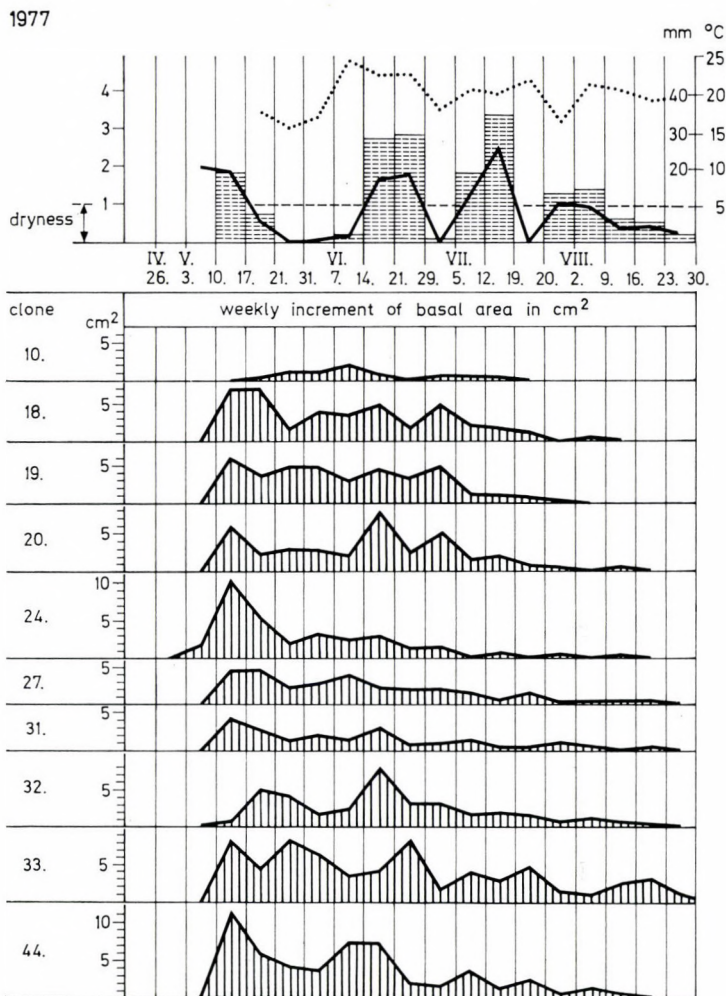


Fig. 4. Weekly basal area growth in cm² in the function of weekly temperatures and precipitation in 1977. — Daily average temperature in °C; === sum of weekly rainfall in mm; — — — values of the formula: $\frac{\text{sum of weekly rainfall in mm} \times 10}{\text{sum of daily average temperatures}}$

Diameter growth of the poplars is generally more intensive in two great periods, from May to the middle of June and from the middle of July to the middle of August.

It can be observed that in the course of the improvement the offsprings obtained by crossing of the same parent trees may have long and short growing periods, too. (E.g. Nos 36, 37 as well as the clones Nos 43, 44.) When the offsprings are studied this circumstance must also be taken into consideration.

The hybrid introduced from Czechoslovakia (clone No. 38) is bursting later than the home-crossed hybrids *P. alba* × *P. tremuloides* (Table 7).

Table 7

A summary made on the basis of the phenological observations of the studied Leuce poplars

| Serial number | Species — clone | | Beginning of | | Duration of "green peak" | Duration of total foliation |
|---------------|---|----------|--------------|-----------|--------------------------|-----------------------------|
| | | | flowering | foliation | | |
| 2. | <i>P. tremula</i> L. | | early | late | average | average |
| 9. | <i>P. canescens</i> Sm | | early | average | average | average |
| 10. | <i>P. canescens</i> Sm | | average | late | short | short |
| 18. | <i>P. canescens</i> × cv. 'bolleana' | H 372-1 | late | late | short | short |
| 19. | <i>P. canescens</i> × cv. 'bolleana' | H 372-2 | late | late | short | short |
| 20. | <i>P. canescens</i> × cv. 'bolleana' | H 372-4 | average | average | rather short | rather short |
| 24. | <i>P. canescens</i> × cv. 'bolleana' | H 428-3 | average | early | rather short | long |
| 31. | <i>P. canescens</i> × <i>P. alba</i> | H 441-8 | late | average | average | average |
| 32. | <i>P. alba</i> L. | | late | average | average | long |
| 33. | <i>P. alba</i> L. | | average | average | average | average |
| 35. | <i>P. alba</i> L. | | average | average | average | long |
| 36. | <i>P. alba</i> × <i>P. tremuloides</i> | H 456-1 | early | early | long | long |
| 37. | <i>P. alba</i> × <i>P. tremuloides</i> | H 456-2 | average | average | average | short |
| 38. | <i>P. alba</i> × <i>P. tremuloides</i> (Spalek) | | average | late | average | average |
| 39. | <i>P. alba</i> × <i>P. grandidentata</i> | H 422-1 | average | average | long | long |
| 40. | <i>P. alba</i> × <i>P. grandidentata</i> | H 422-6 | average | average | long | long |
| 43. | <i>P. alba</i> × <i>P. alba</i> | H 425-4 | average | late | rather short | short |
| 44. | <i>P. alba</i> × <i>P. alba</i> | H 425-10 | average | early | long | long |

Phenological analysis of the poplar clones belonging to the Aigeiros section

Some species and clones, respectively, which are important for the forestry, are given below.

| | Origin |
|--|------------------------|
| 52. <i>P. nigra</i> L. | Czechoslovakia |
| 54. <i>P. ×euramericana</i> cv. I-154 | Italy |
| 55. <i>P. ×euramericana</i> cv. I-214 | Italy |
| 58. <i>P. ×euramericana</i> cv. <i>gelrica</i> | Holland |
| 59. <i>P. ×euramericana</i> cv. <i>regenerata</i> | West-Germany |
| 67. <i>P. ×deltoides</i> ssp. <i>missouriensis</i> Henry | Holland cv. 'Heidemij' |
| 74. <i>P. ×euramericana</i> cv. <i>marilandica</i> | Bátaszék (Hungary) |
| 75. <i>P. euramericana</i> cv. <i>robusta</i> | Belgium |
| 82. <i>P. nigra</i> (hybrid) | Lébény, Hungary |
| 98. <i>P. euramericana</i> | Nagylózs, Hungary |

The elaboration of the Aigeiros poplars was carried out similarly to that of the Leuce poplars but the period of flowering was not considered as important as these poplars are mainly propagated vegetatively. Flowerings generally begin 10 days later than in the case of poplars belonging too the Leuce section.

The beginning of the foliation with the Aigeiros poplars the order can be seen at the Table 8.

Table 8
Phenological data of bursting of the Aigeiros poplars

| Number of clone | Day of bursting from the beginning of the year | | | | |
|----------------------------------|--|------|------|------|---------------|
| | 1976 | 1977 | 1978 | 1979 | average |
| Early-bursting ones | | | | | |
| 67. | 103 | 84 | 111 | 93 | 98 (8. IV.) |
| 82. | 103 | 85 | 109 | 93 | 98 (8. IV.) |
| 55. | 106 | 87 | 105 | 99 | 99 (9. IV.) |
| Poplars bursting at average time | | | | | |
| 54. | 105 | 85 | 109 | 99 | 100 (10. IV.) |
| 75. | 106 | 87 | 111 | 99 | 101 (11. IV.) |
| 74. | 106 | 91 | 111 | 100 | 102 (12. IV.) |
| 52. | 108 | 97 | 111 | 100 | 104 (14. IV.) |
| 59. | 107 | 97 | 114 | 101 | 105 (15. IV.) |
| Late-bursting ones | | | | | |
| 58. | 111 | 108 | 118 | 103 | 110 (20. IV.) |
| 98. | 112 | 109 | 115 | 107 | 111 (21. IV.) |

Table 9
Duration of "green peak" period in days

| Number of clone | Species — clone | 1976 | 1977 | 1978 | 1979 | Average |
|---|--------------------------|------|------|------|------|---------|
| Poplars of a long "green peak" period | | | | | | |
| 55. | 'I-214' | 162 | 182 | 165 | 147 | 164 |
| 67. | 'Heidemij' | 165 | 171 | 159 | 140 | 159 |
| Poplars of an average "green peak" period | | | | | | |
| 52. | <i>P. nigra</i> | 160 | 172 | 138 | 148 | 155 |
| 54. | 'I-154' | 159 | 163 | 146 | 149 | 154 |
| 82. | <i>P. nigra</i> (hybrid) | 165 | 161 | 145 | 143 | 154 |
| 59. | 'regenerata' | 187 | 151 | 137 | 138 | 153 |
| Poplars of a short "green peak" period | | | | | | |
| 75. | 'robusta' | 158 | 159 | 138 | 140 | 149 |
| 74. | 'marylandica' | 158 | 157 | 138 | 136 | 147 |
| Poplars of a rather short "green peak" period | | | | | | |
| 98. | <i>P. × euramericana</i> | 156 | 143 | 139 | 136 | 144 |
| 58. | 'gelrica' | 153 | 140 | 129 | 138 | 140 |

While the duration of foliation from the beginning of bursting to the beginning of defoliation is demonstrated in the Table 9.

This is the period of the volumen-growth or of "green peak" to. Comparing the "green peak" periods of the poplars belonging to the Aigeiros section with those belonging to the Leuce section it can be seen that 'I-214' of long "green peak" period could not be ranged but in the average category of the Leuce poplars.

Table 10
Length of the growing period of various poplars

| Number of clone | Species — clone | 1976 | 1977 | 1978 | 1979 | Average |
|-------------------------------------|--------------------------|------|------|------|------|---------|
| | | day | | | | |
| Poplars of long growing period | | | | | | |
| 55. | 'I-214' | 207 | 223 | 236 | 207 | 218 |
| 67. | 'Heidemij' | 212 | 220 | 233 | 207 | 218 |
| 54. | 'I-154' | 208 | 227 | 205 | 215 | 214 |
| Poplars of average growing period | | | | | | |
| 59. | 'regenerata' | 201 | 195 | 227 | 197 | 205 |
| 52. | <i>P. nigra</i> L. | 202 | 197 | 196 | 209 | 201 |
| Poplars of short growing period | | | | | | |
| 82. | <i>P. nigra</i> (hybrid) | 207 | 203 | 196 | 188 | 199 |
| 74. | 'marylandica' | 191 | 199 | 197 | 190 | 194 |
| 98. | 'euramericana' | 196 | 190 | 197 | 193 | 194 |
| 75. | 'robusta' | 189 | 201 | 182 | 191 | 191 |
| Poplar of very short growing period | | | | | | |
| 58. | 'gelrica' | 181 | 177 | 204 | 180 | 186 |

The order of species from the beginning of bursting to the complete defoliation is compiled in the Table 10.

S. KOHAN in Czechoslovakia, W. MORGENEYER and W. BORS DORF in the DRG also carried out phenological observations. Comparing their observations with our ones, poplars are bursting later both in Czechoslovakia and in the DRG but the order of bursting is similar to the bursting order under home circumstances. It is clear from this that the bursting order depends on the clone but the beginning of vegetation depends on the site and weather influences, respectively.

The comparison between the home and foreign bursting times in case of some Aigeiros clones can be seen in the Table 11.

While a summary of the rate of foliation of the poplar species of the studied Aigeiros section is compiled in the Table 12.

Poplar 'I-214' has the longest growing period. When this clone got to Hungary, its frost sensitive characteristic was considered by many experts as not favourable. This property may derive from that it is bursting early, consequently, a period of frosty weather in March may have a damaging effect on the sap circulation of plants already flushed.

On the basis of the tables the species which begin to come into leaf later can be chosen, thus they can be planted in an area, which is more sheltered from the frost. We established that the poplars of longer and average growing periods generally produce a higher annual increment.

Table 11

Comparison between the home and foreign bursting times in case of some Aigeiros clones

| Species — clone | In days from the beginning of the year | | |
|------------------------------------|---|------------------------------------|---|
| | Czechoslovakia, Eperjes (the average of three years) | GDR (the average four years) | Hungary, Csalános (the average of four years) |
| Early-bursting ones | | | |
| <i>P. deltoides</i> cv. 'Heidemij' | — | 110 | 98 |
| 'I-214' | — | 108 | 99 |
| Poplars bursting at average time | | | |
| 'I-154' | — | 111 | 100 |
| 'robusta' | 116 | 118 | 101 |
| <i>P. nigra</i> | 112 | 109 | 104 |
| 'marylandica' | 125 | 120 | 102 |
| 'regenerata' | 121 | 117 | 105 |
| Late bursting ones | | | |
| 'gelrica' | 125 | 121 | 110 |

Table 12

Summary of the rate of foliation of the poplar species of the studied Aigeiros section

| Number of clone | Species — clone | Beginning of bursting | Duration of | |
|-----------------|--------------------------|-----------------------|-------------|--------------------|
| | | | green peak | complete foliation |
| 52. | <i>P. nigra</i> | average | average | average |
| 54. | 'I-154' | average | average | long |
| 55. | 'I-214' | early | long | long |
| 58. | 'gelrica' | late | very short | very short |
| 59. | 'regenerata' | average | average | average |
| 67. | 'Heidemij' | early | long | long |
| 74. | 'marylandica' | average | short | short |
| 75. | 'robusta' | average | short | short |
| 82. | <i>P. nigra</i> (hybrid) | early | average | short |
| 98. | <i>P. euramericana</i> | late | very short | short |

With the phenological observations of the poplars we tried to answer the question, which phenophase depends on the clone or variety and which are the phenophases that definitely depend on the climate and meteorological influences.

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ÜBER DIE VERBREITUNG, ÖKOLOGIE UND SYNÖKOLOGIE VON *OSTRYA CARPINIFOLIA* IN GRIECHENLAND

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(Eingegangen: 15 Oktober, 1983)

A contribution to the knowledge of *Ostrya carpinifolia* Scop. in special consideration to the synonyms, the vernacular names, namely the new greek, ancient greek and the most important foreign names of this plant, as well as a contribution to the spreading and chorology with specimina graeca (a distribution dot map in the Greek area) and finally to the ecology and synecology of hop-hornbeam is given.

Allgemeines

Ostrya Scop., Flora carniol. 414 (1760) Fam. Corylaceae

Die Gattung *Ostrya* umfaßt nach HESS, LANDOLT und HIRZEL (1967) 3 Arten: *O. carpinifolia* Scop. in Südeuropa und Südwestasien, *O. virginiana* (Mill.) K. Koch u. *O. knowltonii* Coville und nach KRÜSSMANN (1962) 7 Arten in Europa, Asien und Amerika.

Ostrya carpinifolia Scop., Fl. carniol. ed. 2, 2: 244 (1772) (Abb. 1).

Syn.: *Ostrya vulgaris* Willd. (1805), *O. italica* Spach (1841), *O. italica* Scop. ssp. *carpinifolia* (Scop.) H. Winkl. (1904), *Carpinus ostrya* L. (1757), *C. italica* Scop. (1840) in HEGI (1957).

Deutsch.: Gemeine Hopfenbuche. Engl.: European hop-hornbeam, ironwood. Franz.: Ostrie, Charme houblon, Bois de fer. Ital.: Ostria, Carpinella, Carpino nero. Griech.: Ostryá. Oustriá (Pilion, Chalkidiki). Merántsia (Thessalia), Merantza (Akarnania, Epiros, Chalkidiki u. a.). Nerantza. Meliogauros. Skylogauros. Maurogravos (Epiros). Gravos (Thesprotia). Tetramythos (Athos) und Altgriech.: Ostrýa, ostryis oder óstrys (THEOPHRASTOS (?) Pflanzenhistorie 3, 10, 3 in VOLIOTIS und ATHANASIADIS 1971).

Ar. Geogr.: SCHMUCKER 1942: Karte 81, 82; ČERMÁK 1955: 67 und 77; FENAROLI 1967: 129 in JALAS and SUOMINEN (1976).

Blütezeit: IV bis V (VI).

Verbreitung

Das Areal von *Ostrya carpinifolia* Scop. erstreckt sich auf das Mediterran- und Submediterrangebiet von SO.-Frankreich, Korsika und Sardinien bis Bulgarien und bis zum Evros-Fluß in der Europäischen Türkei, nordwärts bis Südösterreich (Flora Europaea 1: 60, 1964; Atlas Florae Europaeae 3: 63, Karte 280, 1976) und weiterhin nach Osten durch Kleinasien ostwärts bis in den Kaukasus und südwärts bis zu den Taurus Ketten (HESS et al. 1967).

Das Gesamtareal der ostmittelmeerisch-submittelmeerischen Art erstreckt sich in Europa auf die Apenninen- und die Balkanhalbinsel, vorwiegend auf Italien mit Sizilien,

Jugoslawien, Albanien und Griechenland und in Vorposten auf die Inseln Korsika und Sardinien, wie auch auf SO.-Frankreich, Schweiz, Österreich—in Ungarn ist sie ausgestorben—Bulgarien wenig auf die SW. europäische Türkei (vgl. BROWICZ 1978).

Die ziemlich beschränkte Verbreitung der reliktschen Art *O. carpinifolia*, die in Europa keine nähere Verwandtschaft hat, ist anscheinend nur auf Gründe der inneren Erschöpfung zurückzuführen, wie auch bei anderen reliktschen Arten der Balkanhalbinsel *Picea omorica*, *Pinus heldreichii* (*P. leucodermis*), *P. peuce*, *Corylus colurna* u. a. (STOJANOV 1930).

In Griechenland wächst die Hopfenbuche besonders in Gebirgsstöcken von Varnous, Voras und Rhodopen bis Taygetos und von den Ionischen Inseln [Kephallinia, Leukas, Kerkira (?)] bis auf die Ägäischen Inseln (Euboea, Thasos und Samothraki) (s. Karte).

Ostrya carpinifolia steigt im montanen Griechenland von den Bergfüßen, wie z. B. von 180 m in der Nähe des Dorfes Pilima von Xanthi oder anderswo von ca. 250 m, wie in Cholomon, Olympos, in der Schlucht des Asopos-Flusses u. a., bis ungefähr 2000 m auf, so in isolierten Gruppen fast unterhalb der sehr steilen, felsigen Gipfel des griechischen Teiles von Ali Botuš. Jedoch liegt die mittlere oberste Höhe des Hopfenbuchen-Vorkommens beträchtlich niedriger. So steigt sie in Olympos bis 1800 m, in Rhodopen und Hagion Pneuma bis ca. 1600 m, in Pindos bis ca. 1500–1600 m, wie z. B. in Wäldern von Pertouli, W-Athamanika-Gebirge (Tzoumerka) u. a., in Voumistos bis 1400 m, in Kerkini und Athos bis ca. 1300 m usw.

Jenseits und in der Nähe der griechischen Grenze kommt *Ostrya carpinifolia* in Nidže Pl. [Voras] (DEGEN und DÖRFLER 1897), Dudica Pl. (Kozur) [Tzena] (SCHULTZE-JENA in BORNMÜLLER 1928a), Dub-Gebirge bei Doiran-See (CIRIMOTIC 1958), Beles (Belasitsa) [Kerkini] bis 1100 m und Ali Botuš, bis 1200 m (STOJANOV in MARKGRAF 1942), stellenweise auch im Bergsystem Rila-Rhodopen, bis 1100–1300 m vor (STEFANOV 1943).

Fundorts-Übersicht

Inter vicum Koula et pagum Psaradhes, 1.–5. 8. 1957, GOULIMIS 406, ATH (GREUTER 1977).

Florina-Gebiet, bei der Warmgrenze der Buchenwälder (MOULOPOULOS 1965).

Kelli (Gorničova Pl.) Mittlere Lage, 500–1000 m. Hagios Athanasios (Ceganska Pl.) 500–800 m (FORMÁNEK 1899).

M. Voras; Paliourda am Nebengießbachbett von Ano Koryfi des Orma-Gießbaches, 680 m und in Dragos, 650 m (MOULOPOULOS 1965). — Gemischtes Strauchwerk des Bergfusses bis zu einer Höhe von 550 (650) m (VOLIOIS 1975). — In gebüschten degradierte Wälder (VOLIOIS 1976a). — Wälder der Therma-Schlucht (Loutra Pozar), 25. 7. 1967 und 15. 6. 1976 (No 1475) mit Früchten; Laubwälder der Gegenden (von Pefcoton 1000–1500 m) von Peteinos-Koukourou u. a. 26. 5. 1974 und 18. 6. 1976 (No 1476) mit Früchten (VOLIOIS 1979). — In Buchenwälder (DROSSOS 1977).

Herbarium Sylvo-Botanicum Universitatis Thessalonikensis.

M. Paikon, bei Dörfern Archangellos, ca. 1200 m und Aesymi, ca. 600 m (HSBUTH).

M. Kerkini (Beles Pl.), Mittlere Lage, ca. 500–1000 m (FORMÁNEK 1900). — Schlucht von Platanakia, 550 m, S. W. Exposition auf Glimmerschiefern, nebst Buche (MOULOPOULOS 1965). — Bei dem Dorf Ano Poroia, 25. 8. 1966 (HSBUTH). — Einzelne Individuen zwischen Buchenwäldern (DROSSOS 1977).

M. Ali Botuš, von den Bergfüßen der Abhänge und der umliegenden Täler einzelne Hopfenbuchengruppen steigen bis ca. 2000 m, fast unterhalb der steilen, felsigen Berggipfel. Auf den NW-Hängen von Mikra Rachi steigt die Hopfenbuche nur bis 1160 m auf, während sie in Südexpositionen des Paliouviri-Gegenhalts, auf unsteilen, Neigungen bis 1250–1350 m (PAPAIOANNOU 1957).

M. Lailias, in Schlucht von Mokra, 980 m, einige Individuen im Buchenmischwald, 11. 7. 1970, fruchtend (VOLIOIS 1976c).

M. Vrontou, im Waldkomplex von Ano Vrontou (GOFAS, pers. Mitt.).

M. Menoekion bei der Buchenwarmgrenze, gegen die Serrae-Vrontou-Strasse Hopfenbuche unter allen gemischt (MOULOPOULOS 1965).

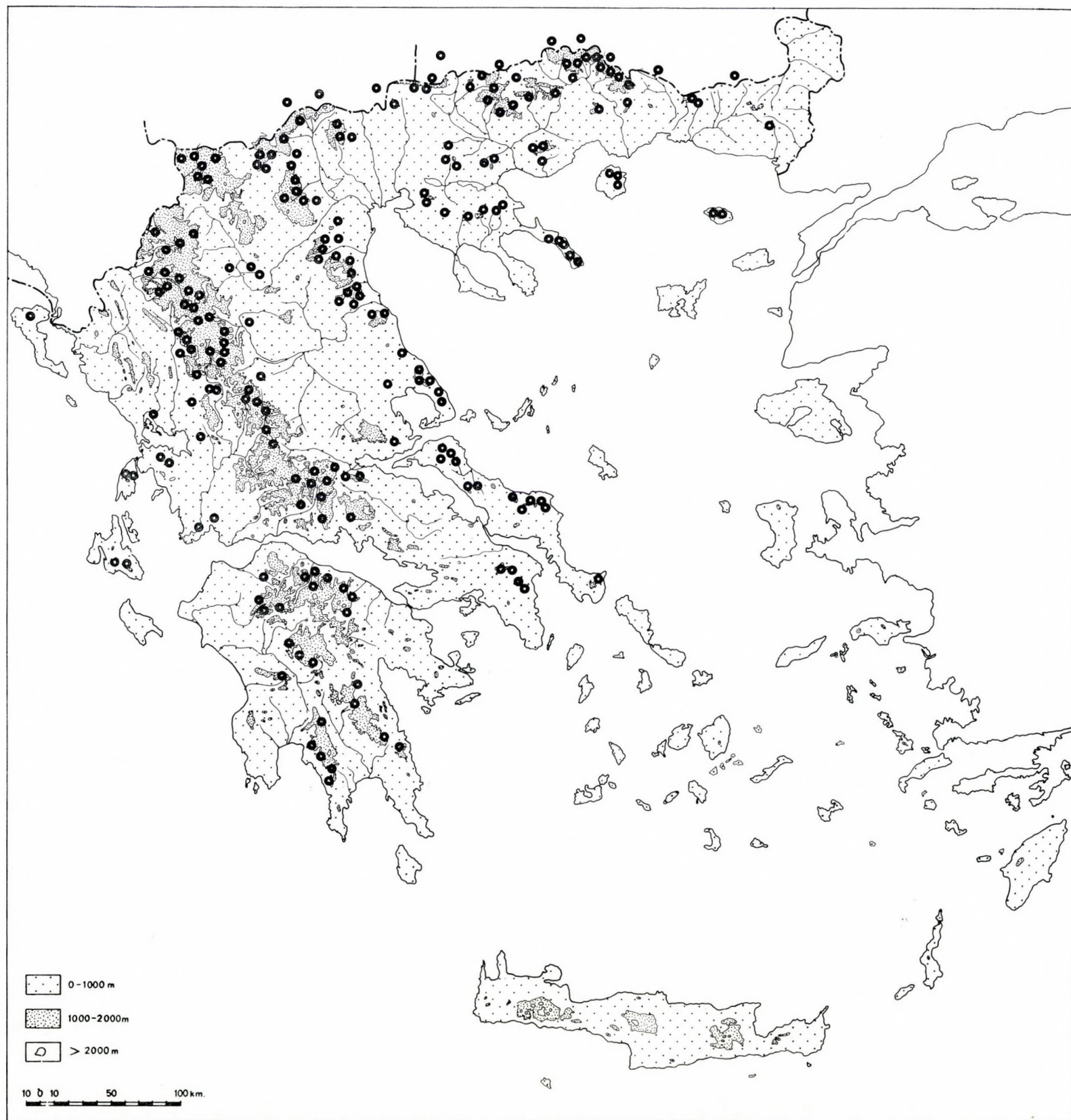


Abb. 1. Verbreitungspunktkarte von *Ostrya carpinifolia* Scop. im griechischen Raum



M. Hagfion Pneuma (Schilka Pl.). 1580 m und M. Falakron (Boz-Dagh), 1200 m, 8. 6. 1941 (KITANOV 1942). — Nördlich Drama, zwischen Livaderon und Taxiarchae, 350 m (ZOLLER et al. 1977). — Kato Neurokopion (GOULIMIS, ATH).

M. Rhodopen: Elatias- und Zagrantenia-Gebiete, 1400–1600 m (SMYRIS, pers. Mitt.). — Drymos (Chaindou)-Gebiet in Mischwäldern (DROSSOS 1977). — Mauri Petra, 1280 m (ZOLLER et al. 1977). Gerakas-Pilima-Gebiet von Xanthi Hopfenbuchen in Buchenwäldern mit anderen sommergrünen Elementen. Chionotopos (Karlik Dere) in der Nähe von Isan-Kagias am der Strasse Symvola (von Komotini)-Mikra Ada bei Buchenwarmgrenze mit Eichen und anderen sommergrünen Gehölze (MOULOPOULOS 1965).

Lekani von Kechrokampos (GOULIMIS, ATH).

M. Pangaeon: Kouri von Mikron Soulion, ca. 1000 m, N. Exposition, im Buchenwald (MOULOPOULOS 1965).

M. Kerkyllia, in Bergschluchten (MOULOPOULOS 1965).

M. Vertiskos (PAVLIDIS, unveröff.).

N. O. Chalkidiki, bei Neochorion und Kryoneri, ca. 500 m (DAFIS 1969).

M. Cholomon: Taxiarchis, Itamos (POLITIS in VOLIOTIS 1967). — In Wäldern spärlich zerstreute und einige vereinzelte Individuen, April 1964 und Aug. 1965 (VOLIOTIS 1967). — Bei Galatista, 200 m, 20. 5. 1967; bei Taxiarchis, 850 m, 7. 6. 1966; bei Arnaea, 950 m, 7. 5. 1966 (HSBUTH).

M. Chortiatis: im Mischlaubniederwald (GANIATSAS 1939a). — In Buchenwarmgrenze, ca. 700 m (MOULOPOULOS 1965).

M. Athos: In Laubwald (REGEL 1942). — Zwischen Iviron und Lavra (MATTFELD 1927) und oberhalb Lavra, 620 m (TURRILL 1937 in RECHINGER 1943). — In Macchien und Mischwälder auf dem Wege von Kapsokalyvia nach Prodromos und Lavra (RAUH 1949). — Quelle von Hagios Athanasios-Lavra, 24. 5. 1950; Filotheou, 3. 5. 1951; Zographou-Karyae, 14. 4. 1952 (GANIATSAS 1963). — In Buchenwäldern der Kloster von Filotheou, Grigoriou, Hagios Paulos und Megisti Lavra, ca. 800–1200 m (MOULOPOULOS 1965). — Im Tal unterhalb der Panagia am Athos-Gipfel, ca. 1250 m (ZOLLER et al. 1977).

Ins. Thasos, Berglagen des N. O.-Teiles der Insel. 1. In Bergschluchten oberhalb von Patamiá, ca. 600 m, Jungindividuen. 2. In *Pinetum pallasianae*. 3. Auf kalkhaltigen, felsigen Boden zwischen oberen Hypsarion und Kamenos Vrachos, 1000–1100 m, einige Sträucher in Lücke felsiger Stücke (STOJANOV und KITANOV 1945: 280).

Ins. Samothraki (KATSIKOPOULOS 1936: 303).

M. Vernon (Vitsi): Moriki-Berg: in Buchenwälder (DROSSOS 1977).

Frequens in sylvis supra Edessa (Vodena) (GRISEBACH 1844). — Pr. Agras, ca. 200 m (HSBUTH).

M. Vermion: Karataš, untere und mittlere Lage, 300–1100 m (FORMÁNEK 1899). — In *Castanetum sativae* und *Fagetum sylvaticae* (GANIATSAS 1939b). — In Buchenstufe, ca. 1200 m (RECHINGER 1939). — Versant oriental du Vermion sur la route de Celi, 750 m. Même localité, 1 km environ au nord du précédent 800 m et 3 km environ en amont du relevé nr 2, 950 m (QUÉZEL 1967). — In *Ostrya-Carpinion orientalis*, 300–1000 m (VOLIOTIS 1975, 1976a). — In Kastanien- und Buchenwälder (DROSSOS 1977). Seli (Karkatsouli) (GOULIMIS, ATH). — Hagios Nikolaos, ca. 450–600 m und Mega Reuma, ca. 650 m (HSBUTH).

Pr. Fytia von Veroea, ca. 450 m (HSBUTH).

M. Vourinos, in Mischwälder (GOULIMIS 1960).

M. Voion: Vythos, 1100 m; Dragasia, 900 m (HSBUTH).

M. Grammos: in valle fl. Vourmbianitikos contra pagum Plikati, 100 m, 13. 8. 1976 (GREUTER 1977, No 14305).

M. Smolikas: in Konitsa und Konitsa-Pades nach Tsotilion (REGEL 1942).

M. Tymfi (Gamila) Scanneli (GOULIMIS 1954). Bei Vrysochori und nach Neraidovrysi; von Konitsa-Brucken nach Moni Stomiou (SFIKAS 1982).

M. Lakmos (Peristeri): inter Chaliki und Kotouri ca. 1150 m und Klinovos in Berg-region bis untere Lage (FORMÁNEK 1896).

M. Athamanika (Tzoumerka): in der Bergregion bei Kalentini, Matsoukion und Kalar-rytae (HALÁCSY 1894). — Gelegentlich erblickt man auf der Westseite des Gebirges bis 1450 m vereinzelte Exemplare (BALDACCI 1897). — Vaptistis, ca. 800 m (HSBUTH). Schlucht des Aaos-Fl. zwischen Tymfi (Gamila) und Trapezitsa (SFIKAS 1980).

Vikos-Schlucht, die Hopfenbuche bildet anderswo sehr dichtes und anderswo spärliches Gebüsch die Schlucht entlang (GANIATSAS 1971).

Ad W. pagi Kranea, in lat. laevo vallis rivi Kira Kali (Paliochori), 1350 m, 16. 8. 1974 CHARPIN 11177, GREUTER et al. 12349 (GREUTER 1977).

M. Pindos, untere Lage und Bergregion von Pincios-Tal, ca. 200 m bis Malakasi, ca. 1200 m und Tannenregion oberhalb Malakasi, 1200–1450 m (FORMÁNEK 1896). — Perivoli

von Grevena, 1300 m; Kalomoera; Gardikion (Monasterion von Panagia), 1000 m; Mesounta von Arta, 1200 m; Vythos, 1100 m und Dragasia, 900 m von Kozani (HSBUTH). — Wald von Pertouli, 1400–1500 m (DAFIS 1975). — Acheloos (Aspropotamos)-Tal, Velistaena und Kastania im Pindos (FORMÁNEK 1898). Bei Kanalia von Karditsa, 400 m und Hagios Nikolaos von Kalampaka (HSBUTH). — Pinde, 3 km à l'ouest de Krania, 1300 m. Entre Pertoulion et Psychi, 1500 m. 3 km avant Psychi, 1300 m. 3 km à l'est et 2 km à l'ouest de Pertoulion, 1550 et 1300 m. Chapelle d'Aghia Paraskevi, 1400 m. 3 km à l'ouest d'Elati, 1550 m. Calcaires (BARBERO und QUÉZEL 1976).

M. Agrafa: Voulgara bei Dörfer Molocha und Neraida, zwischen *Abies borisii-regis*-Wald (DROSSOS 1977). Fourni (GOULIMIS, ATH).

M. Pieria: N.-Expositionen (DIAPOULIS 1940). — Bergregion, 650–900 m (MOULOPOULOS 1965). — Morna-Fteri, 950 m (DAFIS 1969). — In *Ostryo-Carpinion aegaeicum* (VOLIOTIS 1976a). — Ritini, 700 m und Katafygion von Kozani, 1000 m (HSBUTH).

M. Olympos: untere Lage und Bergregion, von 170 m (Ligaria) bis 1200 m (FORMÁNEK 1896). — Olympos (SINTENIS in HALÁCSY 1904). — Abhänge nordwestlich von Litochoron, 400 m. Unter der Zonen von *Pinus nigra* ssp. *pallasiana* und *P. heldreichii* (DIAPOULIS 1936; 1940). — Enipeus-Schlucht (MOULOPOULOS 1965). — Supra vicum Litochoron, ad Monasterium Hagios Dionysios, 850 m, 7. 8. 1973, No 14032 et inter locum Prioni et refugium »Spilios Agapitos«, 1200–1400 m, 8. 8. 1973, Vo 14783 Leg. PHITOS, GEORGIADIS und TZANOUDAKIS (UPA). — In *Ostryo-Carpinion aegaeicum* (VOLIOTIS 1976a). — 2–3 km avant le premier refuge, 700 m, montée par Litochoron, 800 m. 1–4 km après le premier refuge 800–1100 m. 2 km avant la fin de la route forestière, 1120 m. Calcaires (BARBERO und QUÉZEL 1976). — Olympos (DROSSOS 1977). — Ostseite des Gebirges oberhalb Litochoron, 700 m (ZOLLER et al. 1977). — Von Bergfüsse, zwischen 250–800 m bis 1800 m unterhalb von Refugium A STRID 1980).

In valle Tempi (FORMÁNEK 1896; HELDREICH in HALÁCSY 1904; BARBERO und QUÉZEL 1976).

M. Ossa, inzwischen dichten Formationen von *Quercus ilex* (MAVROMMATIS 1971). — In *Ostryo-Carpinion aegaeicum* (VOLIOTIS 1976a). — Ossa (DROSSOS 1977). — Karitsa (Kechria), 800 m, 740 m und 560 m. Seloma, 950 m. Zwischen Seloma und Karitsa, 800 m und 740 m, 5. 7. 1974 (RAUS 1977).

M. Mavrovouni: Paßhöhe südwestl. Keramidi, Hudewälder (RAUS 1977).

M. Pilion (CHLOROS in HALÁCSY 1904 und MAIRE und PETITMENGIN 1908). — Süd-Pilion: Passa Dendro, 620 m; Varsamou, 690 m; Koromilia, 860 m (MOULOPOULOS 1965). — Entre Volos et Portaria, 1100 m. Près de Makrirachi, 300 m. A Aghios Ioannis et 4 km de Aghios Ioannis, 700 m. Près de Tsangarada, 250 m. Schistes (BARBERO und QUÉZEL 1976).

Velestinon in Thessalia (FORMÁNEK in VANDAS 1909). Ins. Euboea (HELDREICH in BOISSIER 1879). — M. Telethron und M. Dirphys (BRETZL in RECHINGER 1943). — M. Xiron, bei Hagia Anna, 750–950 m (RECHINGER 1961). — Kerasia, 600 m (HSBUTH). — M. Kandilion, Schluchten 10 km W. Prokopion (RECHINGER 1961). — M. Xerovouni (TOUNTAS in RECHINGER 1943). — Ibid., in saxosis regionis superioris (HUTH). — Vitala (HSBUTH). — M. Pyxaria (PHITOS 1960a). — 6 km S. Kymi und M. Ochi, 3 km W. Kap Kafireus (RECHINGER 1961).

Nea Kerasous von Prevesa, 350 m (HSBUTH).

M. Makrynoros (GOULIMIS, ATH).

M. Voumistos, 800–1400 m (MAIRE und PETITMENGIN 1908).

M. Arakynthos, in Kleisoura bei Aetolikon (DIMADIS 1916).

M. Tymfristos, bei Karpenision (MATTFELD 1927). — Ibid., 1500–1900 m (BARBERO und QUÉZEL 1976). — Inter opp. Karpenision et pagum Stenoma, ad E. jugi Kokkino Dhiaseio, 11.–15. 6. 1952 (GOULIMIS, ATH in GREUTER 1977).

M. Oxya, Berg Goulinas einzelne Individuen in Eichenwälder (PHITOS 1960b).

M. Vardousia: Artotina, 1200 m (HSBUTH). — In ditone pagi Sykia, ca. 650 m, 20. 6. 1975 No 17782. Leg. PHITOS, KAMARI, PAPATSOU e. a. (UPA). — Entre Artotina et Grammeni Oxya, 900 m. A 8 km de Grammeni Oxya dans le massif du Vardousia, 950 m. Limite inférieure de la Sapinière avant remontée vers Grammeni Oxya, 800 m. Sapinière après Grammeni Oxya, 1450 m. 5 km avant Pentagi — route de Vardousia, 1200 m. Entre Krokyllon et Pentagi, 1000 m (BARBERO und QUÉZEL 1976).

M. Oeta (HELDREICH in HALÁCSY 1904). — Mavrolithari (MAIRE und PETITMENGIN 1908). — Am Oberrand der *Quercion-ilecis*- als auch im unteren Bereich der *Abietion-cephalonicae*-Zone (VOLIOTIS 1976b). — Bei Sklithron, 800 m (HSBUTH).

Descend à 300 m, dans les gorges de l'Asopos, au sud de Lamia- (MAIRE und PETITMENGIN 1908).

Au-dessus de Thermopyles. Calcaires (BARBERO und QUÉZEL 1976).

M. Giona and M. Parnassos (MAIRE und PETITMENGIN 1908). — Parnassos, in sommergrünen Laubwälder (VOLIOTIS 1976b).

1 km à l'est du Lidorikion, 500 m. Flysch (BARBERO und QUÉZEL 1976).

M. Parnis (HELDREICH in HALÁCSY 1904). — Katsimidi (GOULIMIS, ATH).

M. Pentelikon (Penteli), Nordseite, Kümmerexemplaren von *Ostrya carpinifolia* (RIKLI 1946).

M. Erymanthos (Olonos and M. Kyllini (ORPHANIDES in BOISSIER 1879). Erymanthos, in declivibus boreo-orientalibus, ca. 1450 m. in rupestribus calc., 25. 6. 1968, No 8472. Leg. PHITOS (UPA) et supra pagum Kalentzi, ad locum Vathy, ca. 1500 m. 10. 7. 1975, No 2987. Leg. TZANOUDAKIS und STEPHANOU (UPA).

M. Chelmos (HALÁCSY 1904). — Kalavryta, Abhänge am Vouraikos, Kastro, Hagia Lavra, Mega Spilaeon (obs.) (BORNMÜLLER 1928b). — In ditone pagi Aroania, ca. 750 m, 5. 10. 1967, No 13156. Leg. KAMARI (UPA) et in declivibus meridio-orientalibus, ca. 1000 m, 30. 10. 1969, No 11161. Leg. PHITOS (UPA). — A 3–4 km de Kalavryta, route de Patras, 900–1000 m. Route de Bralia (BARBERO und QUÉZEL 1976).

Kyllini-Oligyrtos, adret après le village de Lafka, 1100 m. 200 m après Lafka sous le Kyllini, 950 m. Sapinière de Lafka, 1500 m et au-dessus du village de Lafka, 1480 m. Calcaires (BARBERO und QUÉZEL 1976).

M. Maenalon, Mischwäldern nördlich von Dimitsana, Leukochorion, Lagadia, 700–1000 m (ROTHMALER 1944).

M. Parnon (Malevo) (HELDREICH et ORPHANIDES in BOISSIER 1879). — Aghios Petros, 950 m et peu avant l'embranchement route du Parnon, 1100 m. Region Bambaka, 1100 m. Schistes (BARBERO und QUÉZEL 1976).

M. Taygetos PSARIDES, Jul. 1870, HUTH; HELDREICH et ORPHANIDES in BOISSIER 1879). — Descend à 400 m. dans la Langada de Xirokampi (MAIRE und PETITMENGIN 1908). — In unteren Montanstufe (ZAGANARIS 1934). — Chaos von Lakonia (GOULIMIS, ATH). — Langastra de Trypi à l'est de Sparti, 800 m et près de Trypi, 700 m. Calcaires (BARBERO und QUÉZEL 1976).

Ins. Kephallinia: M. Aenos (HELDREICH in BOISSIER 1879).

Ins. Leukas, zwischen Alexandros und Platystoma, ca. 400 m, wenig unter dem Rand der Doline, N.-Expos. 17. 6. 1966. Auch in der Schlucht nördlich von Kalamitsi (HORMANN 1968).

Ins. Kerkyra (HELDREICH und MILIARAKIS 1925; Betätigungsbedürftig).

Ökologie – Synökologie

Ostrya carpinifolia ist eine halbschatten- bis lichtliebende Holzart. Sie gehört in die Formation der sommergrünen, breitblättrigen Gehölze der griechischen Halbinsel und hat fast die gleiche Verbreitung wie *Carpinus orientalis*. Sie steigt aber höher als diese, ohne bis zur Kaltgrenze der Buche zu gelangen. In der Trockenwarmgrenze bevorzugt die Hopfenbuche halbsonnige Plätze, während sie im Klimax-Verein und an kühleren Stellen im Umwelt wachsen kann. Dort gedeiht sie auf Böden verschiedener Zusammensetzung auch auf Kalkböden, während sie im trockenwarmen Teilareal die trockenen Böden und Topoklimate meidet und sie, wie THEOPHRASTOS richtig erwähnt, zu einem »Wasser- und schluchtliebenden« Baum wird, der hygrophiler als *Quercus pubescens* ist.

Im allgemeinen gedeiht die Art auf trockenen und warmen Abhängen, auf buschigen, felsigen und steinigen, sonnigen, hügeligen Erhöhungen an Waldrändern, -lichtungen und in verlichteten Wäldern auf kalkarmem und kalkreichem Substrat.

In der unteren Stufe wächst die Hopfenbuche in Macchien, Pseudomacchien bzw. Šibljak gewöhnlich in Strauchform, gelegentlich kann sie an geschützten Stellen als ein Baum mittlerer Größe erscheinen. In größeren Höhen nimmt die Art an der Zusammensetzung des Unterholzes verschiedener Laub- oder Mischwälder teil. Diese Wälder bestehen aus Eichen, Kastanien, Buchen (*Fagus sylvatica* und *F. moesiaca* und weniger *F. orientalis*), Tannen (*Abies cephalonica* und *A. borisii-regis*) und Kiefern (besonders *Pinus nigra* ssp. *pallasiana* und *P. heldreichii*). Lokal kann *Ostrya carpinifolia* noch mit folgenden Holzarten beigemischt auftreten: *Quercus pubescens*, *Q. frainetto*, *Q. petraea*, *Q. coccifera*, *Q. ilex*, *Castanea sativa*, *Tilia tomentosa*, *T. platyphyllos* ssp. *platyphyllos*, *Betula pendula*, *Carpinus orientalis*, *Corylus*

avellana, *Fraxinus ornus*, *Acer campestre*, *A. monspessulanum*, *A. platanoides*, *A. pseudoplatanus*, *Ulmus glabra*, *U. minor*, *U. laevis*, *Sorbus torminalis*, *Cornus mas*, *Euonymus latifolia*, *Alnus glutinosa*, *Platanus orientalis*, *Populus tremula*, *Salix caprea*, *Ligustrum vulgare*, *Sambucus nigra*, *Juniperus oxycedrus*, *Taxus baccata* u. a. Gehölzen.

Verschiedentlich gibt es im griechischen Raum Buchenkomplexe, wo man, besonders an der Buchenwarmgrenze, auf die Existenz der Hopfenbuche geradezu wartet, weil diese die Trockenheit mehr als die Buche erträgt und leichter als sie regeneriert. So wächst die Buche im Voras-Grenzgebirge besonders auf Gneis- und Flyschgesteinen außer mit *Ostrya carpinifolia* mit folgenden Hölzern gemischt: *Quercus frainetto*, *Q. petraea*, *Q. cerris*, *Carpinus orientalis*, *Corylus avellana*, *Fraxinus ornus*, *Castanea sativa*, *Tilia platyphyllos*, *Alnus glutinosa*, *Platanus orientalis*, *Salix caprea*, *Betula pendula*, *Acer platanoides*, *A. pseudoplatanus*, *Sorbus torminalis*, *Ilex aquifolium* u. a. (VOLIOTIS 1975, 1976a, 1979; DROSSOS 1977).

Im griechischen Kerkini beteiligen sich an Buchenwäldern auf Glimmerschiefern, ausser *Ostrya* folgende Holzarten: *Quercus pubescens*, *Q. frainetto*, *Q. petraea*, *Carpinus orientalis*, *Fraxinus ornus*, *Cornus mas*, *Sorbus torminalis*, *Alnus glutinosa*, *Platanus orientalis*, *Tilia platyphyllos*, *Ulmus glabra*, *Pyrus amygdaliformis* u. a. (MOULOPOULOS 1965). Östlicher in einem Buchenmischwald vom Lailias-Stock kommen mit *Corylus avellana*, *Sorbus torminalis*, *Cornus mas*, *Euonymus latifolia* u. a. auch einige Individuen von *Ostrya carpinifolia* vor (VOLIOTIS 1976c).

Im benachbarten Waldkomplex von Ano Vrontdou wurden durch ungünstige anthropogene Einflüsse Buche und Eiche aus dem Klimax-Verein verdrängt und die Entwicklung von Hopfenbuche, Orientalische Hainbuche und Haselnusse begünstigt. In den W.-Rhodopen [Drymos (Chaidou)-Gebiet] kommen Mischwälder von *Fagus moesiaca*, *F. orientalis*, *Pinus sylvestris*, *Picea abies*, *Ostrya carpinifolia*, *Betula pendula*, *Corylus avellana*, *Sorbus torminalis*, *Cornus mas*, *Salix caprea*, *Quercus petraea*, *Alnus glutinosa* u. a. vor (DROSSOS 1977). In Mischwäldern von *Fagus sylvatica*, *Pinus sylvestris* mit *Acer campestre*, *Abies alba* und *Picea abies* der bulgarischen Zentralrhodopen wachsen als meistens buschige Holzarten *Ostrya carpinifolia*, *Corylus avellana*, *Populus tremula*, *Salix caprea*, *S. nigricans*, *Cornus mas*, *Alnus glutinosa*, *Betula pendula*, *Sorbus aucuparia*, *Prunus divaricata*, *P. spinosa* u. a. Entsprechende gemischte Wälder, die in tiefen schattigen Schluchten der Ost-Rhodopen (Bu) vorkommen und deren Bestandteile von *Fagus orientalis*, *Ostrya carpinifolia*, *Carpinus betulus*, *Juglans regia*, *Vitis vinifera* ssp. *sylvestris* u. a. sind, werden nach STOJANOV (1930) wohl berechtigt mit tertiären Wäldern verglichen. In den breitblättrigen sommergrünen Wäldern der Athos-Halbinsel wächst die Buche mit *Castanea sativa*, *Ostrya carpinifolia*, *Carpinus orientalis*, *Fraxinus ornus*, *Corylus avellana*, *Quercus ilex*, *Q. pubescens*, *Acer campestre*, *A. platanoides*, *Sorbus aucuparia*, *Prunus mahaleb*, *Cornus mas*, *Euonymus latifolia*, *Alnus glutinosa* u. a. (GANIATSAS 1963; MOULOPOULOS 1965). In Laubgehölzen von Vermion mischt sich die Hopfenbuche mit *Castanea sativa*, *Tilia tomentosa*, *Carpinus orientalis*, *Corylus avellana*, *Fraxinus ornus*, *Acer campestre*, *A. heldreichii*, *A. hyrcanum*, *A. platanoides*, *A. pseudoplatanus* u. v. a. (VOLIOTIS 1975, 1976a; DROSSOS 1977). In den südlicher anschließenden Buchenwäldern von Pieria wachsen auf Glimmerschiefern außer *Ostrya* auch viele andere baumartige und buschige Arten (MOULOPOULOS 1965; VOLIOTIS 1976a). Im Olympos findet man die Hopfenbuche in der untersten Stufe, wo sie z. B. bei Litochoron und im Tempi-Tal, inmitten von sommergrünen Gebüsch und in Macchienv egetation siedelt. Im Unterholz der Laubwälder bildet sie Strauchformen, während sie an günstigen Stellen als ein Baum mit einem mittelgroßen Stammdurchmesser bis 40 cm erscheint. Als Waldbaum wächst sie in gemischten Laub-Nadelwäldern als auch in reinen Kieferwäldern von *Pinus nigra* ssp. *pallasiana* und in der höchsten Stufe von *Pinus heldreichii*, wie z. B. in kompakten Beständen der Kokkinoplos-, Leptokarya- und Sparmos-Wälder (DIAPOULIS 1940; VOLIOTIS 1976a; STRID 1980). Im S.-Pilion, wo die Buche bis an ihre niedrigste Grenze im Bereich des Ägäischen Meeres auf eine

Höhe von ca. 600 m herabsteigt, findet man folgende Gehölze: *Ostrya carpinifolia*, *Quercus pubescens*, *Q. coccifera*, *Q. ilex*, *Carpinus orientalis*, *Fraxinus ornus*, *Acer campestre*, *Taxus baccata* u. a. (MOULOPOULOS 1965).

In das westwärts und südwärts liegende Bergland bis zum Taygetos-Gebirge setzten sich die Vorkommen der Hopfenbuche fort (s. Karte). So kommt sie in der Pindos-Kette von den untersten Lagen, wie z. B. an den Tälern von Acheloos-Fl. und der thessalischen Pinios-Fl. in einer Höhe von ca. 200 m bis zur Tannenstufe vor. In den Wäldern von Pertouli im Zentralpindos wächst die Hopfenbuche mit *Abies borisii-regis*, *Quercus cerris*, *Carpinus betulus*, *C. orientalis*, *Crataegus monogyna* u. a. zusammen (DAFIS 1975). Im Voumistos-Berg erscheint sie auf Kalkböden in Wäldern von *Abies cephalonica* und *Quercus coccifera* (MAIRE and PETITMENGIN 1908). Im Goulinas-Berg von Sterea Hellas (Mittelgriechenland) wächst sie in Eichenwäldern stellenweise und vereinzelt mit *Sorbus torminalis*, *Fraxinus ornus*, *Ilex aquifolium* u. a. zusammen (PHITOS 1960b). In Peloponnisos endlich gibt es *O. carpinifolia* in Mischwäldern der Bergschluchten meist mit *Carpinus orientalis*, *Quercus coccifera* und Ahornsippen (ROTHMALER 1944).

Im pflanzensoziologischen System erscheint *Ostrya carpinifolia* im *Ostryo-Carpinion orientalis* im griechischen Raum bis in mittlere Höhen von etwa 1000 m (VOLIOITIS 1973) und im *Ostryo-Fagion* bis in eine Höhe von 1400 m. In der *Quercion frainetto*-Zone sind außer *Quercus frainetto* auch *Q. dalechampii* sowie Laubmischwaldbestände vertreten. Die letzteren begegnen uns an den nördlichen Hängen mit starker Neigung als Übergang zu *Tilio-Castanetum* mit *Tilia tomentosa*, *Castanea sativa*, *Fagus moesiaca*, *Quercus frainetto*, *Q. dalechampii*, *Ostrya carpinifolia*, *Fraxinus ornus* angesehen werden. Diese Assoziation erscheint nur inselartig (DAFIS 1975). Die Hopfenbuche wächst auch nördlicher in Buschwäldern in der Flaumeichen-Zone (*Quercus-Ostryetum* Horvat 1938). Sie nimmt sogar am *Pinetum pallasianae* teil, wie z. B. auf der Insel Thasos und auf Olympos, auf dem letzteren als *Stachelino-Pinetum pallasianae* Grebenščíkov prov. Hier findet man neben *Pinus nigra* ssp. *pallasiana* in der Baumschicht nicht nur Laubgehölze, wie *Ostrya carpinifolia*, *Quercus pubescens*, *Fraxinus ornus* und *Fagus moesiaca*, sondern auch die nordgriechische Tanne (*Abies borisii-regis*) und die Eibe (*Taxus baccata*). In der Strauchschicht werden ebenfalls submediterrane Arten (z. B. *Buxus sempervirens* und *Juniperus oxycedrus*) und sogar die immergrüne Kermeseiche (*Quercus coccifera*) vertreten (HORVAT et al. 1974). Im Gottesberg steigt die Hopfenbuche noch höher bis zur Nadelwaldzone des *Pinus heldreichii*-Gebiets auf.

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BOOK REVIEWS

Editor: G. FEKETE

WELLS, K.-WELLS, E. K. (editors): *Basidium and Basidiocarp. Evolution, Cytology, Function, and Development*. Springer Verlag New York, Heidelberg, Berlin. 1982. IX + 187 pp. 117 illustrations

The large-scale and ever increasing specialization in recent years, and the mass of information appearing in various, often hardly available journals made it necessary to publish monographs promoting a comprehensive view. The present work in which some major aspects of recent investigations on basidium and basidiocarp are summed up is a case in point.

After a short preface and lists of contents and authors the volume begins with an introduction written by K. WELLS. Chapter 1 deals with the phylogenetic importance of the morphological features of basidium. F. OBERWINKLER, the author of the chapter, discusses the Hetero- and Homobasidiomycetes separately. The primary types are contained in the former. Besides the proved phylogenetic relations the author pays great attention to those not yet sufficiently disclosed to form a starting point from which the further course of phylogenesis can be followed. This particularly applies to the basidial yeasts, and to taxa similar to yeasts.

Chapter 2, on the ultrastructure and cytochemistry of the development of basidium and basidiospores, was written by D. J. McLAUGHLIN. Although the intensive study of the subject started with CORNER's light microscope investigations (1948), electron microscope studies began only in the sixties. Investigations on the ultrastructure were mostly concerned with species of *Aphyllphorales* and *Agaricales*. Cytoplasmic phenomena were studied first of all in species of the genus *Coprinus*. We cannot say, however, that even an approximately satisfactory knowledge of the latter has been acquired so far.

Investigations on the ultrastructure are expected to complete the results of morphological examinations with information on the development of basidia, whereby we might be able to answer, for instance the taxonomic questions of Phragmo- and Holobasidiomycetes. The author gives, though, an account of other authors' researches on the fruiting bodies of various species, but about the initials of basidium formation, the development of the basidial wall, etc. we are informed first of all through the description of studies on *Coprinus cinereus*.

In general, although not exclusively, the development of the basidium is terminal, that is it begins with the swelling of the tip of a hypha. In the teliosporic Basidiomycetes the elsewhere uniform basidium is divided into probasidium and metabasidium. The development of the basidial wall shows a rather wide variation. The development of the probasidial wall in *Uredinales* and *Ustilaginales* is unique. The appearance of septa in the basidia, and the initial phenomena of the development of sterigmata are discussed briefly. Then, the formation of the basidiospores, the differentiation of the cytoplasm and the development of inner membranes and other organella are discussed in detail.

The fusion of the pair of nuclei and the subsequent meiosis take place in the basidium, as proved by several observations. The small size of the nuclei of Basidiomycetes, and the fact that they cannot be stained by the generally used methods explain why their detailed cytological examination began only in the thirties. Ch. THIELKE, the author of Chapter 3, gives an account of investigations he and other authors have carried out concerning the behaviour of the spindle and nuclear membrane during meiosis.

The species of the genus *Coprinus* offer an example of synchronized meiosis not known elsewhere. B. C. LU, the author of Chapter 4, made his investigations on *Coprinus cinereus*.

Of the basidia found on one fruiting body 70–75% are in the same stage of meiosis, which is an obvious advantage, especially in experiments related to DNA replication and genetic recombination.

Since in the *Coprinus* species all laminae of the fruiting body show the same stage of development, there is an opportunity to a continuous check of the course of various phases of the meiosis.

The time required for meiosis at normal temperature (25 °C) can be modified by heat (35 °C).

The effect of temperature on recombination has been studied by genetists. According to the author's experiments heat is efficiently applied at any phase of the meiosis, while cool (5 °C) brings about most recombinations in the pachytene stage. In the author's opinion heat and cooling have a delaying effect on the fusion of the chromosome pairs and increase thereby the change of recombination.

ISAO UNO and TATSUO ISHIKAWA, the authors of Chapter 5, give the title "Biochemical and genetic studies on the initial phase of fruiting body formation" to this chapter. The development of basidiocarp, the fruiting body of the higher Basidiomycetes, postulates the co-ordinated activity of quite a range of genes responsible for the structure and regulating their functioning. Under certain conditions, such as the senescence or injury of the mycelium, or in response to some chemicals the haploid mycelium, may also form fruiting body. The authors undertook the task of studying the role of cycling AMP (adenosine 3',5'-cyclic monophosphate) in starting the fruiting body formation. The cyclic AMP seems to control the glycogen production of the fruiting body-forming mycelium. This compound naturally depends on certain — so far hardly known — enzymatic processes, as regards both its formation and action.

The effect of various parts of the mycelium and the interaction of different mycelia have an important role in the growth of fungi and in the evolution of their organs. In Chapter 6, "The role of pileus and mycelium in the elongation of the stipe in *Flammulina velutipes* and other species of *Agaricales*" by H. E. GRUEN, this question is examined.

If the parts of the fruiting body are of tissue-like inner organization, the physiological connection between them can even be studied macroscopically. The author discusses that phase of the fruiting body formation which is characterized by an increased rate of cell growth. This process culminates when the stipe reaches its final length.

As known from the investigations of a number of authors it is the pileus — and the lamellae in particular — that play an important role in the elongation of the stipe. Experiments of different kind have also been carried out, giving information about the importance of many other factors (light, wave-length of light, periodicity of illumination, role of culture mycelium, etc.); these experiments are, however, far from being regarded as either satisfactory or completed.

A number of growth hormones of vascular plants (indole acetic acid, gibberellin-like substances, compounds with a cytokinin-like action) have been detected in fungi. Nevertheless, according to the author it is not known as yet what role — if any — they play in the fruiting body formation in *Agaricales*.

At the end of the chapter the author summarizes the major results of his partly unpublished experiments with the fruiting bodies of *Flammulina velutipes*. As an interesting conclusion of the experiments the stipe in the stage of intensive elongation no longer depends on the growth hormone production of the lamellae, which otherwise becomes greatly reduced by then. In an earlier stage, on the other hand, it cannot dispense with the growth hormones.

The evolution of the fruiting bodies of mushrooms is of fundamental importance in commercial mushroom production. Studies in this subject may answer questions related to the mass appearance of fruiting bodies and to the effects of factors promoting or hindering it. G. W. GOODAY, the author of Chapter 7, after a brief methodological introduction presents the results of his studies on fruiting bodies of *Coprinus cinereus*.

Analysing the trehalose and glucose content of the stipe the author arrived at conclusions practically identical with those in the literature: in the course of the elongation of the stipe trehalose increases while glucose decreases in quantity. The glycogene in the fungi is a general polysaccharide reserve. The protein content of the elongating stipe appears to be relatively constant, while the increase of the chitin content is a precondition of the longitudinal growth of the stipe.

From the scope of subjects of basidium and basidiocarp those worthies of interest were chosen for the volume. The authors are specialists of the field they undertook to summarize.

The text of the volume is characterized by conciseness. The relatively large number of illustrations (the parts of figures are much larger in number than 117, the number indicated on the front-page), on the other hand, prevent this conciseness from causing difficulties in

understanding. The high typographical level of the work, the exquisite cover are worthy of a volume of the microbiological series of Springer-Verlag. At the end of each chapter a list of references is found, which is helpful in obtaining detailed information.

The volume, which remains in close connection with practical life even when discussing most abstract theoretical questions, is equally recommended to those directing the practice of mushroom production and to researchers engaged in theoretical mycology.

S. TÓTH

Karl-Heinz KREEB: *Vegetationskunde*. Verlag Eugen Ulmer, Stuttgart, 1983. pp. 331.

It seems that Karl-Heinz KREEB cut off more than one can chew when he decided to write a handbook, under the title "Vegetationskunde", aiming at a summarization of the field. He did so in a peculiar, long-lasting developmental stage of Vegetation Science in which more and more diverse views appear and the development of new concepts or at least new methods is apparently a fashionable if not obligatory task of scientists. In this situation the vast literature offers very little motivation for synthesis, the orientation is difficult because one may easily miss the wood for the trees.

The Central European workers are faced with even more difficulties because the traditional Zürich-Montpellier (and Uppsala) school of phytosociology is still active. Thus, one has to be courageous enough in attempting to combine this non-theoretical, but synthesis-oriented approach with the analytical Anglo-Saxon school which strives for a more theoretical basis.

By selecting the title "Vegetationskunde" the author wishes to have this term accepted all over the world. In his views the term Phytosociology (Pflanzensoziologie) has less general validity, being restricted to the floristic background and the arrangement of vegetational units (Pflanzengesellschaft) in a hierarchical system, etc. The task of Vegetationskunde, as expressed in the book, is to delimitate and describe vegetational units keeping in mind their spatial aspects and also considering the causal ecological relationships that are necessary to understand the occurrence of a vegetation type under the given ecological and floristical conditions.

KREEB's book falls into three sections:

General Vegetation Science

Methods of Vegetation Science: The analysis and description of vegetational units

Special Vegetation Science

In the general part is defined the place of Vegetationskunde among the other disciplines of geobotany. The concept of plant community is touched on, a plant community is interpreted as a biological system in relation to the concept of ecosystem. From this point of view are discussed some functional phenomena, such as productivity, and some other modern topics, e.g., stability, types of stability, climax, climax structure, thermodynamics and irreversibility. The presentation of these ideas is interesting by itself, but reading this part the reviewer has the feeling that the consistent overview of the sub-disciplines of vegetation science listed on page 14 (synmorphology, synchorology, synphylogeny, syndynamics, syntaxonomy) would have been more straightforward than the sequence of topics followed by the author. For instance, synphylogeny is ignored here, even if there is some indication of it masked with the title "Ecosystem" on page 26. Syndynamics and, in particular, its important qualitative aspects are not sufficiently detailed and we search in vain for the general principles of syntaxonomy either. Some important issues which would have received more attention in the first part, emerge only per tangentem at the very end of the book (in the Summary). These are, for example, what is a community? how to distinguish between communities? etc.

In the second part (Methods of Vegetation Science) are seen most clearly the difficulties and pitfalls referred to in the introduction. The starting point is offered obviously by the Zürich-Montpellier school. However, it strikes the reader that the BRAUN-BLANQUET approach is degraded to the level of methodology with no explanation. The same note holds true for the so-called "new Russian school". It would have been more adequate to include a chapter under the title "Cenological theories, schools and approaches", or something like that. Its lack is more than obvious. A good and ample source of information for this could have been R. H. WHITTAKER (ed.): *Classification of plant communities* (1980, JUNK).

Picking out special methods is important although secondary. It is questionable whether the "new Russian school" as presented in the book is actually new. The author could have

been advised to use directly one or two fundamental texts by the Russians (e.g., Geobotany by JAROSHENKO or ALEXANDROVA's book) instead of references made through WALTER (1979).

The four sub-chapters (similarity coefficients, correlation coefficient, DE VRIES' plexus diagram, χ^2 -test) discussed separately in the methods section are in fact alternative ways of comparing cenological relevés or of the analysis of species behavior. The descriptions and explanations are somewhat too lengthy and elementary, it would have been sufficient to cite some standard statistical texts (e.g. just to mention books in German, see E. WEBER's books). Following the four sub-chapters, which could have been joined in a single chapter under the title, say, "Similarity", the reader's logic expects some discussion of diversity but this concept is not treated here, nor elsewhere in the book. It remains unanswered why cenological diversity is neglected, as thousands of publications deal with diversity problems. It is to be noted right here that whereas KREEB pays attention to the traditional statistical methods, the information theory functions are not even mentioned in the book. The classification and ordination techniques are commonly used in cenological practice and new methods appear almost every day in the literature. Although classification and ordination were mentioned in the chapter "Complementary methods for the study of vegetation", under a title not emphasizing the importance of the contents, it is not understood why are the clustering techniques so underemphasized. What is the reasoning that standard techniques of multivariate analysis, for example, principal components analysis (PCA), are not discussed either? These are some of the fundamental shortcomings of the book. At the same time, however, there is some "surplus" in the methods section. Manipulations with ecological species groups now represent only supplementary methods in cenology and are by no means equal in importance with the classification and ordination methods. The concept of ecological species groups is closely related to niche theory and the tolerance principle, so that their application is in fact an ecological method.

The third section, Special Vegetation Science, contains two subsections: Phytosociology of Central Europe and The Main Vegetation Types of the World (in that order!). The first is nothing but the description of vegetational units, especially those of Germany. As an introduction, the vegetation of the Tertiary, Pleistocene and Holocene is described and a chronological chapter is provided. The vegetational units are discussed in terms of the principles of the Zürich-Montpellier school, these are alliance (Verband) and orders (Ordnung). References and data are given regarding the functional properties (productivity, mineral nutrient balance, etc.) of certain formations (e.g., forests). The high mountains (Alps), which represent an unique situation, are discussed separately. It is surprising that the dynamic vegetation system of AICHINGER is described right here. Other, skilfully written special chapters (tree line, the origin of meadows and pastures, weed communities, etc.) are outstanding parts of this subsection.

Whereas Central Europe is characterized on floristical basis and by phytosociological methods, the vegetation of other regions of the world are described in much less detail, mainly in terms of the life forms in different formations and physiognomic characters. The principle of classification is climatic and, within the biomes, geographical also. Occasionally, diversified chapters follow one another, for instance, in case of tropical forests: physiognomic structure, taxonomic groups, soil and microclimate, ecophysiology of tropical forests, fauna, etc. In the reviewer's opinion this is the best-written part of the book.

The most emphasized conclusion of the book is that there is no universal method for describing and analyzing vegetation, the high diversity of nature promotes the development of various, often diverging, ideas and views.

Eclecticism is a fundamental character of the book. This could have been hardly avoided without changing the objectives of the book. The data provided are reliable, although not too many references are cited. The selection of literature is biased. The author relies to an extent larger than necessary on standard, comprehensive texts, mainly by German authors, whereas important papers and monographs are neglected. Works by authors from small countries, including monographs on the vegetation of southern Europe, are almost completely ignored even if written in German.

The above criticism does not change the fact that this book is a useful attempt to synthesize Vegetation Science, especially thanks to its well-written parts and the didactic figures. The book is recommended for experts in the field of both botany and ecology.

G. FEKETE

T. F. H. ALLEN and T. B. STARR: *Hierarchy. Perspectives for Ecological Complexity*. The University of Chicago Press, 1982. 310 pages

The simultaneous analysis of several levels of ecological organization relies on hierarchy theory. As this book brilliantly shows, the concept of hierarchic levels is present in many studies even if the authors themselves are not aware of this fact. The general validity of hierarchical organization of nature emphasizes the importance and usefulness of the theoretical framework presented by the authors, both from the University of Wisconsin.

The book falls into three main sections and is supplemented with a glossary. In the Introduction are discussed the middle-number systems that are understood as being composed of too many parts to be adequately described by an equation for each and too few parts to obtain a reliable estimate for their average behavior. These systems are between the large-number systems analyzed by the techniques of multivariate analysis and the small-number systems to which the tools of population biology seem most applicable. As the authors point out, approaches in the domain of large and small numbers are inevitably of limited use and therefore a middle-number level approach is most promising. As suggested, this approach should involve hierarchical models.

The concept of hierarchy is viewed from a historical perspective in the first chapter of Section 1. For the formal investigation of middle-number systems a terminology is developed in the next three chapters, the most important terms being the holon, scale and filter. This rather philosophical section can not be skipped by the reader without risking the intelligibility of the contents.

The theoretical framework of hierarchical organization is developed in Section 2 by discussing various stages of evolution from the abiotic systems to the most complex living systems. Chapter 5 gives a detailed description of nested and non-nested hierarchies. Evolution by natural selection is considered a hierarchical model. In Chapter 6 the nature of boundaries in hierarchical systems is analyzed by distinguishing functional and structural boundaries. Chapter 7 deals with the processes of self-replication. Scaling strategies, as a defensive tool against environmental perturbation, are discussed in the next chapter.

In the reviewer's opinion the last section should deserve the most attention by the practising ecologist. Fundamental problems of the concept, description and analysis of communities are shown to be associated with scale identification. An important point made by the authors is that the identification of the scale of ecological structures should be based on a stepwise change of sample and universe size. They could have gone a little further, however, by pointing out that scale changes are in fact important in any kind of conceptual spaces associated with ecological studies. Further topics discussed in this section include mathematical modelling, diversity and connectedness, microscopic algal ecology and the effect of human impact. These sufficiently illustrate the wide applicability of hierarchy theory. The section ends with a thorough methodological chapter discussing various approaches to the use of scale as an investigative tool.

The book is a readable and up to date summarization of a topic which receives increasing attention by ecologists. The presentation of concepts is quite clear and the text is not free from humour. The only criticism relates to the selection of illustrations, because some of them add nothing to the contents, for example, the picture showing the Windsor post office in Canada. The exceedingly good overview of hierarchical theory makes this book indispensable for those interested in the theoretical and methodological aspects of ecology.

J. PODANI

LANGE, O. L.—NOBEL, P. S.—OSMOND, V. B.—ZIEGLER, H. (eds): *Responses to the Chemical and Biological Environment. Physiological Plant Ecology III*. In the series *Encyclopaedia of Plant Physiology*. Vol. 12 C. Springer-Verlag, 1983. 799 pp.

It is interesting to survey the change that took place in the character of the encyclopaedias during several centuries. That once meant a mere collection and classification of the existing knowledge is today an extremely difficult work of selection requiring strict discipline. This task is carried out excellently and with great care by the authors and editors of the last volume of *Physiological Plant Ecology*. The editors themselves determine the place of the volume in the series as follows: "Growth, development and reproductive success of individual plants depend on the interaction, within tolerance limits, of the factors in the physical, chemical and biological environment. The first two volumes of this series addressed features of the

physical environment (vol. 12 A) and the special responses of land plants as they relate to water use and carbon dioxide assimilation (vol. 12 B). In this volume we consider specific aspects of the chemical and biological environment, and whereas the previous volumes were primarily concerned with the atmospheric interactions, our emphasis here shifts very much to the soil."

The book itself is constructed along a line of increasing complexity so that the first chapter is devoted to the basic mechanisms, while chapters 2-7 deal with the chemical environment, as detailed below.

1. The Ionic Environment and Plant Ionic Relations (M. G. PITMAN, U. LÜTTGE, pp. 30).

This chapter briefly summarizes the processes of ion transport and the participating structures, as variations of a basic mechanism at different stages of organization and evolution. The control of pH and ion content is dealt with in a separate section. The time that had passed after the appearance of the previous two volumes made certain revision and amplification necessary, and although the fundamental importance of the proton motion is invariably emphasized, attention is called to the necessity of a complex view, namely, that the soil environment, the soil-root interaction and the ion transport in the plant should be jointly considered in interpreting the mechanism of control, that the interactions between the different transport processes are important, and that the general model is only a didactic basis of discussion while there is a wide diversity according to the different ecological situations.

2. Osmoregulation (R. G. WYN JONES, J. GORHAM, pp. 24).

3. Halotolerant Eukaryotes (R. MUNNS, H. GREENWAY, G. O. KIRST, pp. 78).

4. Halophytic Prokaryotes (A. D. BROWN, pp. 26).

The effects of ion concentrations are analysed from many aspects, at different levels of organization, with a suggestive exposure of correlations between the component processes. Emphasis is laid on the fact that adaptation is necessary not only to the extent of the environmental stress but also to its variation in time, and that in this respect the capability of adaptation varies with the phases of life. The authors analyse the regulation of ion concentrations and balances, the import-export processes and the compartmentalization as well as their impact on other processes including enzyme-membrane activities, water use efficiency and hydraulic conductance.

5. Physiology and Ecology of Nitrogen Nutrition (M. RUNGE, pp. 38).

In this chapter, too short for importance, the N cycle, N forms and their distribution by season and soil profile, N uptake and conversion, and the relation between N forms and pH are treated. Although M. RUNGE devotes a "special page" to the specific requirements of species, and in one of the subtitles we find the term "in different ecosystems", these aspects have practically been omitted. It is all the more surprising because the author refers to IBP evidences which, however, are not given in detail.

6. Influence of Limestone, Silicates and Soil pH on Vegetation (H. KINZEL, pp. 44).

The author expertly places the soils in different groups according to the availability of univalent and multivalent ions, discussing in detail the different elements and the synergetic effects. Here is — unfortunately hidden in the "inside" of the volume — the fundamental difference in views that separates ecophysiology from the traditional ecology. For that reason a special geobotanical and a special ecophysiological terminology have to be used, and the diversity of habitats and that of the plant responses separately discussed. H. KINZEL finds the solution of the problem in the extension of a comparative experimental work and the consequent establishment of so-called physio-types. It is all the more surprising that the word "niche" is not even mentioned.

7. Toxicity and Tolerance in the Responses of Plants to Metals (H. V. WOOLHOUSE, pp. 56).

The closing chapter of the section discussing the effect of chemical environment deals with the physiological and biochemical mechanisms of metal toxicity and tolerance, while surveying all metals that can be taken into consideration, and confronting the two sides of the phenomenon, namely the "chemism", the special individual characteristics of metals on the one hand, and the molecular biology (mainly enzymatic and membrane changes) on the other. As a special merit of the chapter the evolutionary and genetic implications of metal tolerance are also discussed.

8. Ecophysiology of Nitrogen-Fixing Systems (A. H. GIBSON, D. C. JORDAN, pp. 90).

Owing to its importance in practice the bacterium-root symbiosis is a thoroughly investigated field of research. However, the approximately one thousand works published in the subject, while covering all aspects from genetic engineering to economic utilization, give account only of very few case studies made in natural situation.

9. Ecophysiology of Mycorrhizal Symbioses (M. MOSER, K. HAZELWANDTER, pp. 31).

Within the subject so far rather neglected for its importance, the authors analyse the varieties of the individual mycorrhizae and the structures thereof, and beyond the physio-

logical correlations they deal with the ecological functions of the root-bound fungi which affect the growth characteristics and distribution of the host plant and its resistance to diseases.

10. Ecophysiology of Lichen Symbiosis (U. MATTHES, G. B. FEIGE, pp. 46).

"... a lichen is a micro-ecosystem by itself." This view is emphasized by the authors who take this functional integrity as a basis on establishing the physiological types, choosing the nature of the transfer product as the basis of typification.

11. Interactions between Plants and Animals in Marine Systems (W. HÖLL, pp. 30).

12. Ecophysiology of Carnivorous Plants (U. LÜTTGE, pp. 30).

13. Host-Parasite Interactions in Higher Plants (P. R. ATSATT, pp. 38).

14. Virus Ecology — "Struggle" of the Genes (A. J. GIBBS, pp. 22).

15. Ecophysiology of Zoophilic Pollination (S. VOGEL, pp. 66).

16. Physiological Ecology of Fruits and Their Seeds (D. H. JANSEN, pp. 32).

These chapters have the common feature of discussing the ecological and evolutionary importance of biotic interactions which have so far belonged to the world of extraordinary, bizarre, paradoxical biological forms, often not only from the viewpoint of natural history but also from that of physiology.

17. Physiological and Ecological Implications of Herbivory (S. J. MCNAUGHTON, pp. 22).

An interesting aspect of the herbivore-plant relation is shown by the author who says that while at an ontogenetic level the herbivores are harmful to the plant and many protection mechanisms develop against them, these protection mechanisms never function perfectly and so in ecological time herbivores are useful as demographic regulators and promoters of recyclization, and from this contradiction a peculiar phenomenon of coevolution comes out.

18. Interactions between Plants (E. I. NEWMAN, pp. 30).

If modern encyclopaedia writing requires self-command and a certain scientific asceticism, then NEWMAN wins the palm. The chapter written by him with a high, almost axiomatic precision is remarkable also as regards its conciseness and critical view. The chapter is divided into three parts: methods, mechanisms (and interactions between mechanisms), and the ability of interference, as an ecological strategy.

As to the whole of the volume, the structure of each chapter as well as the sequence of chapters are logical and clear. The abundant references (some 20 per cent of the volume) and the large number of reviews among them allow for a moderate treatment of examples, which is a basic condition of readability and lucidity. It is true, on the other hand, that every author feels the burden of the complexity of his task and the resulting difficulties of interpretation.

The basic conception of the volume is in any case that we must leave behind the physiological standpoint of studying only the general, average basic structures and processes, since the very step that ought to be taken is to assess and typify the enormous diversity appearing in the phenomena discussed. Here we come up, however, against difficulties raised by the methods that are laborious and not easy to apply even in the case of fast plants examined thoroughly, so how could they then be suitable for extensive serial examinations?

This programme, though spectacular and far-reaching, must in any case be criticized!

Namely, the primary ecological objective to be set is to understand why certain definite forms appear at the level of a given association (and not at the mostly autecological level of the book), rather than to assess and describe in detail the diversity of the adaptation types and mechanisms!

And this is positively a problem of niche theory. Thus, the task of physiological plant ecology should be to study the physiological background of the organization (composition and pattern rearrangement) of tolerance niches. Owing to its different view "ecological plant physiology" would be a better title for the present book. Nevertheless, all these problems are too general to put the blame on the authors of the volume. It is the volumes of this very series that take steps to bridge the gap between the two extreme views (physiological and ecophysiological on the one hand, and ecological on the other), and bring them closer to each another.

S. BARTHA

F. LAMBERTI, J. M. WALLER, N. A. VAN DER GRAAFF: Durable Resistance in Crops. Plenum Press, New York, 1983. 454 pp.

Plant diseases and pests are a major constraint to agricultural production despite the various means used to control them. Chemical control, although often effective, may pose environmental hazards and is expensive, especially in developing countries where it may be completely uneconomic.

Control through genetically mediated resistance to diseases and pests is both cheap and environmentally safe and at present most diseases of staple food crops are controlled through some form of resistance.

One of the basic problems in the use of resistance is its frequent lack of durability. The temporary nature of resistance, due to the development of new strains of pest or pathogen able to overcome it, has seriously hindered the improvement of the yield potential of many crops as a continuing effort is needed to replace old cultivars whose resistance has failed, with new ones.

The Food and Agricultural Organisation of the UN began its International Programme on Horizontal Resistance in 1975 with the objective of accumulating high levels of supposedly durable resistance. Considerable interest and scientific debate was generated by these programs which necessitated the meeting and interaction of those concerned. FAO and the International Society for Plant Pathology decided to organize a Conference at which recent results of these programs could be communicated and the various strategies which had been developed could be adequately discussed. This Conference was held in Martina Franca, Italy from September 30–October 11, 1981.

This volume contains the lectures and contributions of this Conference. The presentations often generated extensive discussions much of which are included in the Proceedings as an integral part of them. At the end of the volume the reader finds the list of participants and lecturers of the Conference and a Subject Index.

The 38 Proceedings are grouped into three main sections:

- I. Durable plant resistance and the genetics of host/pathogen populations,
- II. Parameters and targets of durable resistance.
- III. Breeding for durable resistance and its practical application.

The most frequently involved crops are: barley, cotton, fig, grape, coffee, coconut, oil palm, potato, wheat, rice and Faba beans.

On the other hand, the main pathogens discussed are: *Puccinia hordei*, different kinds of Mildew, nematodes, *Fusarium* spp., coffee leaf rust and Berry disease, wheat rusts, *Phytophthora* spp., etc.

One presentation deals with the resistance of Elm, Pine and Cypress species and the related problems from the point of view of Forest Pathology.

The lectures reviewed many theoretical subjects, proposed models for the Host/Pathogen system, as well as practical results of research projects and breeding programs.

As in any relatively new field of science, the ideas are many and diverse, but the practical selection, breeding and management strategies in which they result differ much less.

Questions were raised on the genetic background, specificity, durability, and modelling of resistance; in estimation of pathogen fitness, biometric analysis, and host-pathogen-environment interactions of durable resistance.

At the same time, this volume offers a wide spectrum of practical results and experiences concerning a series of crops and pathogens in every major agricultural area.

To sum it up, this book could provide a useful reference point and in many cases practical help, too in the quest for durable resistance for all crops at any environment. Therefore it can be recommended for every scientist and experts engaged with the problems of resistance of ever increasing importance.

Z. Szócs

M. H. ZIMMERMANN: Xylem structure and the ascent of sap. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1983. 143 pp., 64 figures

This well-edited book is the first volume of a new Springer series in wood science. This is the work, where the renowned publisher M. H. ZIMMERMANN provides first an integrated description of sap ascension from an anatomical and functional point of view.

The volume can be divided into three parts regarding the subject matter. The first two chapters are mostly dealing with the intricate anatomical structure of the xylem. The main part of this work (Chapters 3–4) is really functional anatomical, regarding the cohesion theory of sap ascent and the hydraulic architecture of plant. In the third part (Chapters 5–7) are discussed the other functional adaptations, failure of xylem function and the pathology of xylem.

The first chapter begins with the conducting units, introduced by the evolution and specialization of tracheids and vessels. It is continued by the vessel dimensions and the difficulties of measuring. Different methods are introduced to measure vessel-length distribution. Functional parameters are discussed here such as flow rate, pressure gradient, hydraulic conductivity and the efficiency of vessels — how to move the water through the intervessel pits.

The second chapter is devoted to the vessel network in dicotyledon and monocotyledon stems. The author gives a short outline of methods for the analysis of vessel networks. Water movement and vessel network of some species are introduced, most detailed being the palm stem (*Rhapis excelsa*, a small palm).

The main part of this work starts with Chapter 3. It is really functional anatomical part dealing with the cohesion theory of sap ascent. Here are treated functional parameters such as the tensile strength of water, tension limits, water storage, sealing concepts, pressure gradients and velocities. The section on storage of water is not intended to be a general review on water storage in plants, but concerns primarily storage mechanisms that are more or less directly related to water movement. The following mechanisms are discussed: elasticity of tissues, capillarity, and cavitation.

One of the most interesting part of this book discusses the hydraulic architecture of plants (Chapter 4). In hydraulic considerations the relationship of leaf mass and wood production, has been studies connected with "HUBER value", named after HUBER by the author. After ZIMMERMANN deals with the leaf-specific conductivity (LSC). The name originated from the author (1978), he reevaluated HUBER's record about the effective conductivity.

The hydraulic construction of trees is a very interesting part of this volume. Here the reader can find how LSC values are related to pressure gradients and flow velocities. Comparisons of LSC are made of main stems of ring-porous species and measurements are made to investigate the anatomical basis of the characteristic LSC distribution within tree stems.

"The subject matter of this book is multi-dimensionally related in so many different ways that it is difficult to accomodate it logically in a one-dimensional, linearly proceeding text representation. The organisation of the subject material into chapters and sections has therefore been somewhat arbitrary. Frequent cross references help to restore some of the multidimensionality" as written by M. H. ZIMMERMANN.

Chapter 5, under the heading "Other functional adaptations", has thus become rather a collection of odds and ends. Here the author discusses the radial water movement in the stem which has relatively little experimental evidence till now, speaking about the radial direct and indirect contacts, growth-ring bridges (BOSSHARD 1976). Variations of anatomical characteristics from roots to twigs, the distribution of tracheid dimensions in coniferous trees, the vessel diameters of woody dicotyledons, the variation in length of tracheids and vessels are also discussed. After the function of the wall sculptures and scalariform perforation plates are discussed. An independent part of this chapter deals with the xylem transport in aquatic angiosperms.

Chapter 6 deals with the failure and senescence of xylem function, beginning with the embolism and the winter freezing of xylem water in trees. Here are discussed different methods to investigate how trees of cold climates cope with the problem of winter freezing. After this follows a literature survey about the tyloses, gums, suberization and the heartwood formation and its relation to water transport.

The last chapter (7) is devoted to the pathology of the xylem. M. H. ZIMMERMANN felt necessary to build a bridge between those who were concerned with xylem structure and the ascent of sap, and plant pathologist interested in xylem malfunction. The chapter is not a review of pathological disturbance of water relations in general. Here are concerned the ascent of sap and its disturbance. Wetwood formation, movement of pathogens, the effect of disease on supply and demand of xylem water, xylem blockage, pathogen effects on xylem differentiation and the problem of injecting liquids are discussed.

The writer has no intention to be encyclopedic, although the number of citations is over 400. This volume contains different chapters mainly on various experimental approaches. There are excellent graphs, diagrams, drawings and scanning electron micrographs of high quality, supporting the theoretical aspects.

It is an ideal book that looks at old problems in new ways and highlights fascinating areas of current research, tries to build a bridge between the study area of structure and that of function. M. H. ZIMMERMANN's book is warmly recommended to every specialist and institute dealing with experimental botany.

D. KOVÁTS

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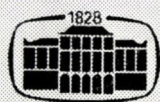
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